

## PHARMACOLOGIST'S REVIEW

PLA: 96-1433

SPONSOR: Genetics Institute, Inc.

PRODUCT: recombinant human interleukin-11; rhIL-11; IL-11; NEUMEGA®; oprelvekin

FORMULATION/CHEMISTRY: Produced in *E. coli* & non-glycosylated, with a MW of ~19 kD and is composed of [~] amino acids. The protein differs from native IL-11 [~]

(b)(4)

~~~~~ } The formulation is as a sterile, nonpyrogenic, lyophilized cake with 23 mg glycine USP, 1.6 mg dibasic sodium phosphate heptahydrate USP, 0.55 mg monobasic sodium phosphate monohydrate USP, pH 7.0, 5 mg/mL.

PROPOSED INDICATION: For the prevention of chemotherapy-induced thrombocytopenia

ABBREVIATIONS: recombinant human interleukin-11, rhIL-11, NEUMEGA®, oprelvekin = IL-11; subcutaneous = SC; intravenous = IV; platelet = PLT; bone marrow = BM; megakaryocyte = MK

assigned 12/20/96; completed 5/27/97

CROSS-REFERENCES: IND #4751

### Table of Contents

|                                   |       |
|-----------------------------------|-------|
| Preclinical Pharmacology .....    | p. 02 |
| PK/ADME Studies .....             | p. 19 |
| Acute Toxicity Studies .....      | p. 28 |
| Multidose Toxicity Studies .....  | p. 29 |
| Reproduction/Teratology Studies . | p. 45 |
| Mutagenicity Studies .....        | p. 50 |
| Conclusion .....                  | p. 51 |

### INTRODUCTION:

IL-11 is a thrombopoietic growth factor produced by fibroblasts, as well as by bone marrow stromal cells, and is also known as adipogenesis inhibitory factor. IL-11 directly stimulates the proliferation of hematopoietic stem cells & megakaryocyte progenitor cells, as well as lymphoid cells. IL-11 also induces the differentiation of megakaryocytes into platelets. Platelets produced in response to IL-11 are normal morphologically & functionally, as well as possessing normal life spans. IL-11 has shown thrombopoietic activity in vivo animal models of compromised hematopoiesis.

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IL-11 is rich in proline [~] leucine [~], & positively charged residues (14%), resulting in a basic cytokine, with an

isoelectric point of 11.7. Under normal conditions, circulating IL-11 is undetectable, but levels are elevated in patients with severe/immune thrombocytopenia. IL-11 belongs to a family of cytokines including IL-6, CNTF, leukemia inhibitory factor (LIF), & cardiotropin (CT) that use the signal transducing receptor subunit gp130 as part of the high affinity receptor complex. IL-11 first binds to the  $\alpha$ -chain & this complex then associates with gp130.

The proposed clinical indication for IL-11 is in the prevention of chemotherapy-induced thrombocytopenia & the reduction of the need for platelet transfusions in patients with non-myeloid malignancies. The proposed package insert submitted by the sponsor states that 50  $\mu$ g/kg/dose NEUMEGA® is to be SC injected once daily 6-24 hours after chemotherapy completion for 10-21 days per treatment course.

Investigational Formulations - Clinical studies were started using a liquid formulation containing 20 mM L-histidine & 0.3 M glycine, pH 7.0. To enhance stability, a lyophilized form [with 20 mM L-histidine & 0.3 M glycine] was then used. This was followed by a third formulation - a lyophilized form containing 10 mM sodium phosphate, 0.3 M glycine, pH 7.0 - further enhancing the stability of the product.

No preservatives are in the formulation, thus the vials are intended for single use only. The formulation contains no human- or animal-derived protein stabilizer. The primary excipient is glycine, which increases the thermal stability of IL-11, as well as protects the protein from vigorous shaking. This commercial product will be manufactured at Parke-Davis.

### Preclinical Pharmacology Studies

#### In vitro

##### List of Studies:

Note that the dates presented with each study are the dates the report was issued, not the date of study completion. The list of studies are numbered in the order in which they appear in the submission.

1. Direct Effects of IL-11 on Megakaryopoiesis in Cell Culture; study #P96079-14; performed at GI; 6/96

33. IL-11 Receptor Expression & the Effects of rhIL-11 on a Colon Carcinoma Cell Line, COLO 320DM; report #DSMCB-001-95; performed at GI; 7/95

34. Interaction of IL-11 with Rat Intestinal Epithelial Cells in Vitro; report #MCB-001-94; performed at GI; 11/94

35. rhuIL-11 Does not Stimulate Tumor Colony-Forming Units from Cancer Patients In Vitro; report #DSPR-001-95; performed at GI; 9/95

36. Interaction of rhuIL-11 with Cytotoxic Cancer Therapies In Vitro & In Vivo Against Human HT-29 Colon Carcinoma; report #PR-001-95; performed at Dana Farber Cancer Institute; 9/95

37. Interaction of rhuIL-11 with Cytotoxic Cancer Therapies In Vitro & In Vivo Against Murine EMT-6 Mammary Carcinoma; report #PR-013-95; performed at Dana Farber Cancer Institute; 9/95

41. Effect of rhuIL-11 on TNF $\alpha$  Production by LPS-Stimulated Murine Macrophage Cell Line RAW264.7; report #DSMCB-002-94; performed at GI; 11/94

42. Effect of rhuIL-11 on Purified Human Neutrophils In Vitro; report #P96088-14; performed at GI; 9/96

#### In vivo

##### List of Studies:

Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

The list of studies are numbered in the order in which they appear in the submission.

1. Effects of IL-11 on Megakaryocytopoiesis & PLT Production in Normal Mice; report #PB-039-91; performed at GI; 10/91

2. Effects of IL-11 on Megakaryocytopoiesis & PLT Production in Splenectomized Mice; report #PB-041-91; performed at GI; 10/91

3. IL-11 Stimulates Multilineage Hematopoietic Recovery in Mice After a Myelosuppressive Regimen of Sublethal Irradiation & Carboplatin; report #PBM-001-93; performed at GI; 6/95

4. The Effects of IL-11 on Normal Rats; report #PB-003-91; performed at GI; 4/91

5. Dose Response & Hematologic Effects of a SC Administration of IL-11 in the Dog; report #DSPR-002-95; performed at GI; 5/95

6. Efficacy of IL-11 in Radiation-Induced Marrow Aplasia & Marrow Transplantation: Stage 1-Pilot Dosing in & Hematopoietic Effects on Nonirradiated Dogs; report #PB-018-93; performed at Fred Hutchinson Cancer Center; 12/93
7. Effects of IL-11 on Hematology Parameters in Nonhuman Primates; report #PB-001-91; performed at Arthur D. Little; 3/91
8. Effects of IL-11 & rhuG-CSF on Mobilization of Hematopoietic Progenitors to Peripheral Blood in Nonhuman Primates; report #PR-015-94; performed at Arthur D. Little; 10/94
9. Effects of SC Dosing of IL-11 in Nonhuman Primates; report #PB-010-91; performed at Arthur D. Little; 6/91
10. Effects of SC Administered IL-11 & rhuG-CSF on Mobilization of Hematopoietic Progenitors to Peripheral Blood in Nonhuman Primates; report #PR-004-95; performed at Arthur D. Little; 8/95
11. Platelet Level Response After SC Administration of IL-11 in Nonhuman Primates; report #PB-017-92; performed at Arthur D. Little; 2/93
12. I. Hematologic Effects of 1-, 2-, or 4-Day SC Administration of IL-11 Followed by Three Single Weekly Doses in Nonhuman Primates...and...II. Hematologic Effects of 4-Day SC Administration of IL-11 (Phase 1 & 2 Clinical trials Formulation) in Nonhuman Primates; report #PB-016-92; performed at Arthur D. Little; 2/93
13. Effects of SC Administration of IL-11 or rhGM-CSF Alone, or IL-11 in Combination with IL-3 or rhGM-CSF in Nonhuman Primates; report #PB-042-91; performed at Arthur D. Little; 10/91
14. Effects of SC Administered IL-11 on PLT Reactivity & PLT Ultrastructure in Nonhuman Primates; report #P96077-14; performed at GI; 10/96
15. Analysis of Plasma Proteins of Nonhuman Primates Treated with IL-11; report #PB-019-91; performed at Arthur D. Little; 8/91
16. Effects of IL-11 on Hematopoietic Reconstitution in Transplant Mice: Acceleration of Recovery of Peripheral Blood Neutrophils & PLTs; report #PB-011-92; performed at GI; 6/92
17. IL-11 Stimulates Multilineage Hematopoietic Recovery in Mice After a Myelosuppressive Regimen of Sublethal Irradiation & Carboplatin; report #PBM-001-93; performed at GI; 6/95

18. Effects of IL-11 on Normal Dogs & After Sublethal Radiation; report #P96118-14; performed at Fred Hutchinson Center; 10/96 [Nash, RA., et. al., Effects of rhIL-11 on normal dogs and after sublethal radiation. Exp. Hematol. 23:389-396, 1995.]
19. The Effects of IL-11 in Myelosuppressed NonHuman Primates; report #PB-021-91; performed at Memorial Sloan-Kettering; 8/91
20. Effects of IL-11 in Myelosuppressed NonHuman Primates: SC Once Daily Administration; report #PB-046-91; performed at Memorial Sloan-Kettering; 10/91
21. Thrombopoietic Activity of IL-11 in a Novel Myelosuppressed NonHuman Primate Model; report #P96048-14; performed at BioDevelopment Labs; 4/96
22. Dose Response & Hematologic Effects of a SC Administration of IL-11 in the Dog; report #DSPR-002-95; performed at GI; 5/95
23. Efficacy of IL-11 in Radiation-Induced Marrow Aplasia & Marrow Transplantation: Stage I - Pilot Dosing in & Hematopoietic Effects on Nonirradiated Dogs; report #PB-018-93; performed at Fred Hutchinson Center; 12/93
24. Effects of IL-11 on the Plasma Volume of Male Splenectomized New Zealand White Rabbits; report #P96091-14; performed at GI; 9/96
25. Acute Effects of IL-11 on Renal Function in Beagle Dogs; report #P96080-14; performed at GI; 8/96
26. A Pharmacology Study of IL-11 (SC) Administered with or without Lasix® (IV) for 6 Days in the Beagle Dog, Followed by an 8-Day Recovery Period; report #54451; lot #2846-161 (lyophilized); performed at Bio-Research Labs; 6/95
27. Effects of IL-11 in the Rat Langendorff Coronary Perfusion Model; report #PH 267-GTI-001-94; performed at Pharmakon USA; 3/95
28. Potential Arrhythmogenic Effects of IL-11 in the Anesthetized Guinea Pig; report #PH 1096-GTI-001-94; performed at Pharmakon USA; 2/95
29. Potential Effects of IL-11 Upon Arrhythmia and Fibrillation Threshold Doses in the Anesthetized Ouabain-Intoxicated Guinea Pig; report #PH 262-GTI-001-95; performed at Pharmakon USA; 2/95
30. Potential Effects of a Test Article in the Anesthetized Ouabain-Intoxicated Guinea Pig; report #PH 262-GTI-002-95; performed at Pharmakon USA; 10/95

31. Hemodynamic Effects of Acute IV Administration of IL-11 in New Zealand White Rabbits; report #PB-048-91; performed at Arthur D. Little; 10/91
32. The Cardiovascular Response of IL-11 Treated Beagle Dogs to IV Infusion of Histamine Dihydrochloride; report #P96085-14; performed at Arthur D. Little; 10/96
38. Alteration in the Frequency, Severity, & Duration of Chemotherapy-Induced Mucositis in Hamsters by IL-11; report #PRM-001-95; performed at GI; 6/95
39. Dose Response & Dosing Schedule Studies of rhuIL-11 in HLA-B27 Transgenic Rats; report #PR-019-94; performed at GI; 11/94
40. Dose Response & Scheduling Studies of rhuIL-11 in Acetic Acid-Induced Colonic Injury in SD Rats; report #PR-018-94; performed at Biodevelopment Labs; 11/94

### Pharmacology Studies

#### In vitro

**1. Direct Effects of IL-11 on Megakaryopoiesis in Cell Culture**  
Low density mononuclear cells were isolated from the BM of normal or 5-FU-treated C57BL/6 mice & mature hematopoietic cells depleted. Lin(-)Sca-1(+)c-kit(+) stem cells were stimulated (blast & colony formation) when cultured with IL-11 + IL-3 + murine lit ligand. MK colony formation was enhanced. RNA for IL-11 receptor  $\alpha$ -chain was detected in the CD41a population of early & late BM progenitors, but not in human or murine PLTS, suggesting that IL-11 can directly interact with MKs.

**33. IL-11 Receptor Expression & the Effects of rhIL-11 on a Colon Carcinoma Cell Line**

In vitro exposure of COLO 320DM cells to 10 or 100 ng/mL IL-11 did not alter their proliferation rate & did not protect the cells from radiation cytotoxicity.

#### **Comment:**

● RT-PCR assays have detected IL-11 receptor  $\alpha$  chain RNA in normal & carcinogenic (colon, mammary) murine tissue, as well as in human carcinoma cell line COLO 320DM - suggesting the potential for direct interaction of IL-11 with cells of the GI tract.

**34. Interaction of IL-11 with Rat Intestinal Epithelial Cells in Vitro**

Nontransformed rat IEC cell lines (IEC-6, IEC-17 - from the small intestine) were grown with/without IL-11 (1, 10, 100 ng/mL) or TGF $\beta$ 1 (10 ng/mL). Exposure to IL-11 resulted in a reduction in the rate of cellular proliferation, as the doubling time was increased to 30 hours with 100 ng/mL IL-11 vs. 20 hours for untreated cells. IL-11 treated cells showed delayed entry into S phase compared to untreated cells, & a prolongation of the G0/G1 phase. TGF $\beta$ 1 also reduced the rate of cellular proliferation & delayed entry into S phase.

**35. rhuIL-11 Does not Stimulate Tumor Colony-Forming Units from Cancer Patients In Vitro**

Fresh human tumors [various types] cultured with 1, 10, or 100 ng/mL of IL-11 showed stimulation in 2/66, 2/66, & 1/66 tumors, respectively. IL-11 appeared to have a concentration-dependent inhibition of growth of human tumor colony-forming units for melanoma, prostate, brain, stomach, carcinoid, breast, ovarian, & non-small cell lung tumors.

**41. Effect of rhuIL-11 on TNF $\alpha$  Production by LPS-Stimulated Murine Macrophage Cell Line RAW264.7**

Murine RAW264.7 cells were treated with 5 ng/mL LPS, with/without IL-11 (1, 10, 100, 500 ng/mL) & the level of induction of TNF $\alpha$  RNA measured by Northern analysis. Data showed that stimulation of the cells by LPS caused elevated TNF $\alpha$  RNA levels & IL-11 did not affect these levels. IL-11 alone did not induce TNF $\alpha$  RNA expression. The amount of TNF $\alpha$  antigen was reduced by 50% for cells treated with LPS & 100 ng/mL IL-11 [potential regulation of TNF $\alpha$  production by a post-transcriptional mechanism].

**42. Effect of rhuIL-11 on Purified Human Neutrophils In Vitro**

Neutrophils from normal subjects were purified & incubated with 100 ng/mL IL-11 for 3 hours, followed by addition of LPS or Zymosan A. Stimulated neutrophils produced IL-8, which was not affected by pretreatment with IL-11 [IL-10 or dexamethasone blocked LPS-induced IL-8 production & dexamethasone blocked Zymosan A-induced IL-8 production. A low level of  $\alpha$ -chain RNA was found in neutrophil RNA - possibly reflective of low IL-11 binding & signaling, not a downregulation of the activation of neutrophils.

In vivo**1. Effects of IL-11 on Megakaryocytopoiesis & PLT Production in Normal Mice**

SC injections of IL-11 at 75  $\mu\text{g/kg/dose}$ , bid, for 7 days to normal C57Bl/6 mice resulted in stimulation of MK production & an increase in peripheral PLTs (maximum of 133% on day 7), compared to control. PLTs returned to baseline by day 15. Increased numbers of CFU-MEG were noted in the BM & in the spleen on day 7.

**2. Effects of IL-11 on Megakaryocytopoiesis & PLT Production in Splenectomized Mice**

SC injections of IL-11 at 75  $\mu\text{g/kg/dose}$ , bid, for 7 days to splenectomized C57Bl/6 mice resulted in stimulation of MK production & an increase in peripheral PLTs (maximum of 141% on day 7), compared to control. PLTs returned to baseline by day 10. Increased numbers of CFU-MEG were noted in the BM on days 3 & 7.

**3. IL-11 Stimulates Multilineage Hematopoietic Recovery in Mice After a Myelosuppressive Regimen of Sublethal Irradiation & Carboplatin**

Mice were administered a combined regimen of sublethal irradiation & carboplatin, followed by SC injection of 250  $\mu\text{g/kg/dose}$  of IL-11 (duration unknown) resulted in a reduction of PLT & HCT nadirs and a reduced period of thrombocytopenia & anemia.

**4. The Effects of IL-11 on Normal Rats**

SC injections of IL-11 at 5, 15, 50, or 150  $\mu\text{g/kg/dose}$ , bid, for 7 days to normal SD rats (kill on day 16) resulted in a dose-related increase in peripheral PLTs (maximum of 34% of baseline by day 7/9), compared to control. PLTs returned to baseline by day 14. Increased numbers of MKs were noted in the BM & in the spleen on day 16.

**5. Dose Response & Hematologic Effects of a SC Administration of IL-11 in the Dog**

SC injections of IL-11 at 50, 88, or 136  $\mu\text{g/kg/day}$  for two cycles of 14 & 12 days separated by a 17-day recovery period, to normal beagle dogs resulted in the following changes:

TABLE 1 Mean Percentage Change Relative to Baseline in the rhIL-11-Treated Group

| Dose                 | Platelet Counts |         | Red Blood Cells |         | Fibrinogen |         |
|----------------------|-----------------|---------|-----------------|---------|------------|---------|
|                      | Cycle 1         | Cycle 2 | Cycle 1         | Cycle 2 | Cycle 1    | Cycle 2 |
| 50 $\mu\text{g/kg}$  | + 69 %          | + 44 %  | - 24 %          | - 17 %  | + 162 %    | + 109 % |
| 88 $\mu\text{g/kg}$  | + 88 %          | + 40 %  | - 26 %          | - 17 %  | + 398 %    | + 64 %  |
| 136 $\mu\text{g/kg}$ | + 134 %         | + 53 %  | - 18 %          | - 18 %  | + 198 %    | + 51 %  |

Parameters returned to baseline within 6-10 days of nontreatment.

#### 6. Efficacy of IL-11 in Radiation-Induced Marrow Aplasia & Marrow Transplantation: Stage 1-Pilot Dosing in & Hematopoietic Effects on Nonirradiated Dogs

Ten (2/grp) normal beagle dogs were SC injected with IL-11 at 30, 60, 120, or 240  $\mu\text{g/kg/day}$ , bid, for 14 days. Dose-related PLT increases were seen [1.6-fold (30 & 60  $\mu\text{g/kg}$ ) and 2.6-fold (120 & 240  $\mu\text{g/kg}$ ) baseline]. No changes in WBCs or BM histology occurred. CFU-GM [mobilization of late hematopoietic progenitors] were increased in blood & BM; serum fibrinogen (1.3-3.3-fold baseline) & cholesterol were increased & albumin decreased (6-30% baseline). The ploidy number of MKs was increased at day 7 & survival of  $^{51}\text{Cr}$ -PLTs did not change. One 120  $\mu\text{g/kg}$  dog died (from pneumonitis) on day 15. Although the pulmonary histopathology was indicative of an infectious process, lung & blood cultures were negative & lung stains for bacteria & fungi were also negative. Baseline and day 1 [the only day evaluated] histamine levels were elevated in the dog that died, compared to the surviving 120  $\mu\text{g/kg}$  dog [note that the dog also had roundworms, which may have caused the histamine elevation].

#### Comment:

- The sponsor acknowledges that the death of the dogs may have been secondary to IL-11 effects. The relationship between thrombocytosis, thromboembolism, and the pulmonary hemorrhages noted should be questioned.

#### 7. Effects of IL-11 on Hematology Parameters in Nonhuman Primates

Continuous IV infusion of IL-11 at 10 or 30  $\mu\text{g/kg/day}$  occurred for 7 days (cyno monkeys followed for 28 days). PLT peaks of ~83% above baseline were noted on day 12/14 at 10  $\mu\text{g/kg}$  & ~145% above baseline on day 14 at 30  $\mu\text{g/kg}$ . PLTs returned to baseline by day 23 for 10  $\mu\text{g/kg}$ , but were still ~20% above baseline for 30  $\mu\text{g/kg}$ . Red cell indices were decreased.

**Comment:**

- Note that the IL-11 receptor  $\alpha$ -chain primary amino acid sequence most closely resembles the IL-6 & the CNTF receptor  $\alpha$ -chain.

**8. Effects of IL-11 & rhuG-CSF on Mobilization of Hematopoietic Progenitors to Peripheral Blood in Nonhuman Primates**

Continuous IV infusion of IL-11 [0.3 M glycine, 20 mM histidine] at 100  $\mu\text{g/kg/day}$  or SC injection of 10  $\mu\text{g/kg/day}$  of G-CSF occurred for 7 days (cyno monkeys followed for 20 days). CD34+ cells/mL increased 1.5-3.8-fold with IL-11 and 5.7-61.2-fold with G-CSF. CFU-GM/mL increased 3-4-fold with IL-11 and 36-62-fold with G-CSF. PLTs peaked at 162% above baseline on day 13 with IL-11, while G-CSF animals showed a nadir of 34% below baseline on day 10. WBCs (neutrophils) increased with G-CSF, but not with IL-11.

**9. Effects of SC Dosing of IL-11 in Nonhuman Primates**

SC injections of IL-11 at 60  $\mu\text{g/kg/day}$ , qid or bid, occurred for 7 days (cyno monkeys followed for 23 days). Data showing increased PLTs, decreased red cell mass & albumin were similar for both dosing regimens.

**10. Effects of SC Administered IL-11 & rhuG-CSF on Mobilization of Hematopoietic Progenitors to Peripheral Blood in Nonhuman Primates**

SC injection of IL-11 [0.3 M glycine, 20 mM phosphate] at 50  $\mu\text{g/kg/dose}$ , bid and/or SC injection of 10  $\mu\text{g/kg/day}$  of G-CSF occurred for 7 days (cyno monkeys followed for 20 days). CD34+ cells/mL increased 1.9-4.4-fold with IL-11; 15.3-132.5-fold with G-CSF; and 12.8-70.9-fold with IL-11 + G-CSF. CFU-GM/mL increased 3-27-fold with IL-11; 61-141-fold with G-CSF; and 56-86-fold with IL-11 + G-CSF. PLTs peaked at 123% above baseline on day 11 with IL-11 & 68% above baseline on day 13 with IL-11 + G-CSF; while G-CSF animals showed a nadir of 40% below baseline on day 11. WBCs (neutrophils) increased with G-CSF, but not with IL-11.

**11. Platelet Level Response After SC Administration of IL-11 in Nonhuman Primates**

Cyno monkeys were SC injected with 100  $\mu\text{g/kg/day}$  IL-11 until PLTs were  $\geq 600 \times 10^3/\mu\text{L}$ . Mean day 10 values were  $853 \times 10^3/\mu\text{L}$ , reaching peak levels on day 12 ( $934 \times 10^3/\mu\text{L}$ ), & approaching baseline by day 20. [Dosing ended on day 9.]

12. I. Hematologic Effects of 1-, 2-, or 4-Day SC Administration of IL-11 Followed by Three Single Weekly Doses in Nonhuman Primates...and...II. Hematologic Effects of 4-Day SC Administration of IL-11 (Phase 1 & 2 Clinical trials Formulation) in Nonhuman Primates

I. Cyno monkeys were SC injected with 60 µg/kg/day IL-11 for 1, 2, or 4 days, followed by additional doses on days 8, 15, & 22. Peak PLT values were 34% increased from baseline on day 8 (1-day dose); 27% increased on day 12 (2-day dose); 82% increased on day 10 (4-day dose). PLTs returned to baseline by day 15 for all animals. Decreased red cell indices & an acute-phase reaction were noted.

II. Cyno monkeys were SC injected with 60 µg/kg/day IL-11 clinical formulation for 4 days. Peak PLT values were 74% increased from baseline on day 10, returning to baseline by day 22. Decreased red cell mass indices were noted (recovered by day 10).

13. Effects of SC Administration of IL-11 or rhGM-CSF Alone, or IL-11 in Combination with IL-3 or rhGM-CSF in Nonhuman Primates  
SC injection of IL-11 at 60 µg/kg/day for 14 days alone or in combination with IL-3 (10 µg/kg/day, for 7 days); GM-CSF (5 µg/kg/day for 5 days); or GM-CSF alone resulted in increased PLT counts:

- 130% of baseline for IL-11 - day 15
- 168% of baseline for IL-11 + IL-3 - day 11
- 209% of baseline for IL-11 + GM-CSF - day 10
- 161% of baseline for GM-CSF - day 10

Decreased red cell indices & an acute phase reaction (day 15) occurred in all IL-11 groups & increased WBCs were noted in the IL-3 & GM-CSF groups.

14. Effects of SC Administered IL-11 on PLT Reactivity & PLT Ultrastructure in Nonhuman Primates

PLTs were obtained from cyno monkeys SC injected with IL-11 at 125 µg/kg/day for 7 days. A 156% PLT increase from baseline was seen on day 11. PLTs retained a normal discoid shape (determined by ultrastructural analysis). Via P-selection upregulation in response to thrombin (PLT reactivity), a slight increase in PLT reactivity after 4 days of IL-11 was noted - correlating with new PLTs in the circulation.

**15. Analysis of Plasma Proteins of Nonhuman Primates Treated with IL-11**

Plasma isolated from cyno monkeys from studies PB-001-91, PB-010-91, PB-017-91, showed evidence of acute phase proteins (total protein, albumin,  $\alpha$ 1-antitrypsin, transferrin,  $\beta$  lipoprotein, haptoglobin, C3, C4, IgG, IgA, IgM, properdin factor B).

**16. Effects of IL-11 on Hematopoietic Reconstitution in Transplant Mice: Acceleration of Recovery of Peripheral Blood Neutrophils & PLTs**

C3H/HeJ mice underwent total body irradiation, followed by BMT & SC injection of IL-11 (100-500  $\mu$ g/kg/dose, for 18-28 days). Recovery of WBCs (neutrophils) was accelerated, reaching normal levels by day 14. CFU-GM derived from both BM & spleen, were increased, as were PLTs.

**17. IL-11 Stimulates Multilineage Hematopoietic Recovery in Mice After a Myelosuppressive Regimen of Sublethal Irradiation & Carboplatin**

SC injection of 250  $\mu$ g/kg/day of IL-11 to myelosuppressed mice increased the number of MK, erythroid, granulocyte, & macrophage progenitors compared to controls. [No other details presented].

**18. Effects of IL-11 on Normal Dogs & After Sublethal Radiation**  
[Note that the data below on normal dogs are identical to study #6]

Ten (2/grp) normal beagle dogs were SC injected with IL-11 at 30, 60, 120, or 240  $\mu$ g/kg/day, bid, for 14 days. Dose-related PLT increases were seen [1.6-fold (30 & 60  $\mu$ g/kg) and 2.6-fold (120 & 240  $\mu$ g/kg) baseline]. No changes in WBCs or BM histology occurred. CFU-GM [mobilization of late hematopoietic progenitors] were increased in blood & BM; serum fibrinogen (1.3-3.3-fold baseline) & cholesterol were increased & albumin decreased (6-30% baseline). The ploidy number of MKs was increased at day 7 & survival of  $^{51}$ Cr-PLTs did not change. One 120  $\mu$ g/kg dog died (from pneumonitis) on day 15. Although the pulmonary histopathology was indicative of an infectious process, lung & blood cultures were negative & lung stains for bacteria & fungi were also negative. Baseline and day 1 [the only day evaluated] histamine levels were elevated in the dog that died, compared to the surviving 120  $\mu$ g/kg dog [note that the dog also had roundworms, which may have caused the histamine elevation].

Five dogs were irradiated with 200 cGy, followed by injection of 240  $\mu\text{g/kg/day}$  IL-11, bid, for 28 days. Death occurred in 2/5 IL-11 dogs & 1/10 control [irradiated] dogs from hemorrhagic pneumonia. The lungs had severe vascular congestion & hemorrhage with infiltrating neutrophils. Culture could not confirm an infectious etiology. The IL-11 dogs exhibited low PLT counts for a median of 24 days compared to 28 days for controls.

#### 19. The Effects of IL-11 in Myelosuppressed NonHuman Primates

Cyno monkeys treated with 5-FU were SC injected with IL-11 at 60  $\mu\text{g/kg/day}$ , bid, for 14 days. Only 1/3 IL-11 monkeys survived; the others died of sepsis. PLT & CFU-GM (BM) recovery were faster in the IL-11 monkey compared to the control monkey. There was no difference in transfusion requirements between the animals.

#### 20. Effects of IL-11 in Myelosuppressed NonHuman Primates: SC Once Daily Administration

Cyno monkeys treated with 5-FU were SC injected with IL-11 at 60  $\mu\text{g/kg/day}$  for 14 days. Only 1/3 IL-11 monkeys survived; the others died of sepsis. PLT data were variable in the study, thus conclusive statements are not possible.

#### Comment:

- Interpretation of the studies in 5-FU treated monkeys were questionable due to many deaths from bacterial infections, the unexpected tolerance of a placebo treated monkey to 5-FU, and the variability of PLT recovery in the IL-11 treated animals.

#### 21. Thrombopoietic Activity of IL-11 in a Novel Myelosuppressed NonHuman Primate Model

Myelosuppression was induced in cyno monkeys via IV injection of carboplatin (days 1-3), with SC injection of IL-11 [10 mM phosphate, 0.3 M glycine] at 125  $\mu\text{g/kg/day}$  either concurrently (day 1) or following chemotherapy (day 4) = Cycle 1. Cycle 2 consisted of SC injection of IL-11 (day 32) following chemotherapy (days 29-31). G-CSF was SC injected at 10  $\mu\text{g/kg/day}$  on day 32, until the ANC was  $>500$  cells/ $\mu\text{L}$ . In Cycle 1, PLT counts for 5/6 IL-11 monkeys did not go below 20,000/ $\mu\text{L}$ , regardless of the treatment schedule [one animal dosed starting in day 1 experienced notable thrombocytopenia]. However, 5/6 control monkeys were at or below this level. In addition, IL-11 treatment showed a range of 2-5 days [day 1 dosing commencement] & 0-3 days [day 4 dosing commencement] for PLT recovery to  $\geq 50,000$  cells/ $\mu\text{L}$ , while controls displayed a range of 3-8 days.

In Cycle 2, 3/3 monkeys injected with IL-11 on day 1 (Cycle 1) & 1/3 monkeys injected with IL-11 on day 4 (Cycle 1) were below 20,000/ $\mu$ L. Controls were at this nadir in 4/6 monkeys. PLT recovery was notably slower in the day 1-cycle 1 dosing regimen compared to the day 4-cycle 1 dosing regimen. Additionally, the time to recovery to  $\geq 100,000$  cells/ $\mu$ L post-cycle 2 was increased for the IL-11 animals compared to controls (see figures).

The day 1-cycle 1 monkeys exhibited a trend toward longer neutrophil recovery times, displayed in cycle 2, compared to the control & day 4-cycle 1 monkeys. Red cell indices were lower for the IL-11 monkeys in cycle 1, but appeared similar to controls in cycle 2. A total of 1/6 control, 2/3 day 1-cycle 1, and 2/3 day 4-cycle 1 animals required RBC transfusions. A total of 5/6 IL-11 monkeys produced anti-IL-11 antibodies, which did not seem to affect/correlate with hematological recovery.

Figure 1 Mean Platelet Counts  $\pm$  SEM for Cycle 1 and Cycle 2  
Control (Group 1) vs. rhIL-11 Treatment (Group 2)

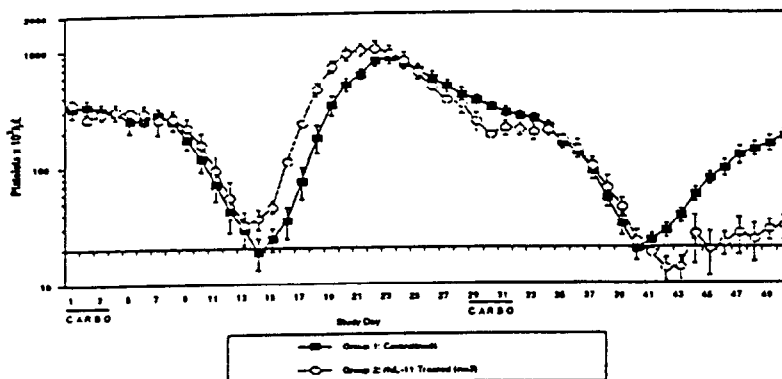


Figure 1. Carboplatin = 25 mg/kg on Days 1, 2, 3, and 29, 30, 31  
rhIL-11 = 125  $\mu$ g/kg (QD) starting on Day 1 (Cycle 1) and continuing until platelets  $\geq 100,000$  cells/ $\mu$ L  
rhIL-11 = 125  $\mu$ g/kg (QD) starting on Day 32 (Cycle 2) and continuing until platelets  $\geq 100,000$  cells/ $\mu$ L  
rG-CSF = 10  $\mu$ g/kg (QD) starting on Day 32 and continuing until ANC  $\geq 500$  cells/ $\mu$ L.

Figure 2 Mean Platelet Counts  $\pm$  SEM for Cycle 1 and Cycle 2  
Control (Group 1) vs. rhIL-11 Treatment (Group 3)

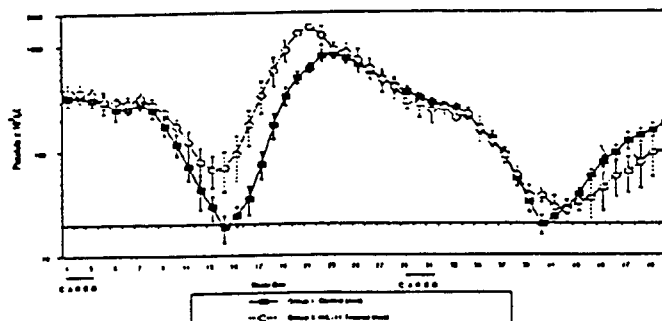


Figure 2. Carboplatin = 25 mg/kg on Days 1, 2, 3, and 29, 30, 31.  
rhIL-11 = 125  $\mu$ g/kg (QD) starting on Day 4 (Cycle 1) and continuing until platelets  $\geq 100,000$  cells/ $\mu$ L.  
rhIL-11 = 125  $\mu$ g/kg (QD) starting on Day 32 (Cycle 2) and continuing until platelets  $\geq 100,000$  cells/ $\mu$ L.  
rG-CSF = 10  $\mu$ g/kg (QD) starting on Day 32 and continuing until ANC  $\geq 500$  cells/ $\mu$ L.

## 22. Dose Response & Hematologic Effects of a SC Administration of IL-11 in the Dog

Dogs were SC injected with IL-11 at 50, 88, or 136  $\mu\text{g/kg/day}$ , in two cycles of 14 & 12 days, separated by a 17-day wash-out period. Resulting data are shown below:

TABLE 1 Mean Percentage Change Relative to Baseline in the rhIL-11-Treated Group

| Dose                 | Platelet Counts |         | Red Blood Cells |         | Fibrinogen |         |
|----------------------|-----------------|---------|-----------------|---------|------------|---------|
|                      | Cycle 1         | Cycle 2 | Cycle 1         | Cycle 2 | Cycle 1    | Cycle 2 |
| 50 $\mu\text{g/kg}$  | + 69 %          | + 44 %  | - 24 %          | - 17 %  | + 162 %    | + 109 % |
| 88 $\mu\text{g/kg}$  | + 88 %          | + 40 %  | - 26 %          | - 17 %  | + 398 %    | + 64 %  |
| 136 $\mu\text{g/kg}$ | + 134 %         | + 53 %  | - 18 %          | - 18 %  | + 198 %    | + 51 %  |

## 23. Efficacy of IL-11 in Radiation-Induced Marrow Aplasia & Marrow Transplantation: Stage I - Pilot Dosing in & Hematopoietic Effects on Nonirradiated Dogs

Same study as study #18.

Nash, R., et. al., Effects of rhIL-11 on normal dogs & after sublethal irradiation. Exp. Hematol. 23:389-396, 1995.

## 24. Effects of IL-11 on the Plasma Volume of Male Splenectomized New Zealand White Rabbits

On day 0, plasma volume was estimated in 10 rabbits using Evan's Blue (injected into the marginal ear vein) & a dye dilution technique. Rabbits were then IV injected with IL-11 at 200  $\mu\text{g/kg/day}$  for 7 days. [Compared to controls] plasma volume increased ~25% by day 2, remaining elevated until day 7. No edema was noted. Red cell indices were also decreased.

## 25. Acute Effects of IL-11 on Renal Function in Beagle Dogs

Anesthetized beagle dogs were IV infused with IL-11 [10 mM phosphate, 0.3 M glycine] at 50  $\mu\text{g/kg}$ . Some dogs were given a 100 mL sodium challenge 1 hour after IL-11 dosing & followed for 3 hours. No arrhythmias or changes in baroreceptor function [MAP/heart rate] occurred in any dog. Transiently increased sodium excretion occurred early postdose IL-11, followed by decreases towards the latter part of the 3 hours - associated with early increases in urinary nitric oxide, followed by decreases in response to sodium challenge. Potassium excretion decreased in response to IL-11 + sodium challenge. IL-11 increased water conservation in all groups - notably in the sodium challenge groups, correlating to the decreased red cell indices seen.

**Comment:**

● Clinical ADRs have included fatigue, atrial arrhythmias, & syncope. The sponsor postulated that these ADRs occurred from the development of dilutional anemia caused by plasma volume expansion. The sponsor notes that basal levels of nitric oxide are produced in the urinary tubules which inhibit Na<sup>+</sup>K<sup>+</sup>-ATPase. Inhibition of nitric oxide synthase results in increased sodium & water absorption in the kidney. IL-6 is known to further induce this system.<sup>1</sup>

**26. A Pharmacology Study of IL-11 (SC) Administered with or without Lasix® (IV) for 6 Days in the Beagle Dog, Followed by an 8-Day Recovery Period**

Beagle dogs (8/sex/group) were SC injected with IL-11 at 0 (saline) or 50 µg/kg/day, alone or with Lasix® (2 mg/kg/day, IV) for 6 days + 8 days recovery. Lasix® was injected ~1 hour after IL-11 injection. There was no effect on clinical signs, body weights, food & water consumption, ECGs, creatinine clearance, or fecal excretion. Other findings:

↑ WBCs (neutrophils, monocytes); APTT; PLTs - IL-11 +/- Lasix®

↓ red cell mass - IL-11 alone (trend toward recovery in ♂s); IL-11 + Lasix® during recovery period

↑ glucose - IL-11 alone

↓ K<sup>+</sup>, Cl<sup>-</sup> - IL-11 +/- Lasix®

↓ urine K<sup>+</sup> & Cl<sup>-</sup>, osmolality; ↑ urine volume, Na<sup>+</sup> excretion, aldosterone - IL-11 + Lasix®

All values returned to baseline.

**27. Effects of IL-11 in the Rat Langendorff Coronary Perfusion Model**

Hearts were excised from rats, followed by retrograde perfusion with Krebs alone or with IL-11 at 15, 50, 150, or 500 ng/mL at a rate of ~4-12 mL/min. Bipolar ECGs were taken continuously & heart rate was obtained at 5-min intervals, along with other cardiac measurements. Patent Blue Violet dye (0.1%) was injected into the hearts & reperfusion verified.

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<sup>1</sup> Trepicchio, WL., et. al., IL-11 attenuates the inflammatory response through down regulation of proinflammatory cytokine production & nitric oxide release. Blood. ASH abstract.

No biologically significant differences in heart rate, coronary blood flow, or ventricular extrasystole were noted and no ventricular tachycardia or fibrillation occurred. Reperfusion of the heart occurred in all groups.

#### **28. Potential Arrhythmogenic Effects of IL-11 in the Anesthetized Guinea Pig**

The jugular vein, carotid artery, & trachea of anesthetized ♂ guinea pigs were cannulated & IV infused (1 min) with IL-11 at 0, 0.1, 0.3, 1.0, 10, or 20 mg/kg. No biologically significant differences in total incidence of arrhythmia & fibrillation, as well as mean arterial pressure changes were noted in the IL-11 pigs compared to controls.

#### **29. Potential Effects of IL-11 Upon Arrhythmia and Fibrillation Threshold Doses in the Anesthetized Ouabain-Intoxicated Guinea Pig**

The jugular vein, carotid artery, & trachea of anesthetized ♂ guinea pigs were cannulated & IV infused (1 min) with IL-11 at 0, 0.1, 0.3, 1.0, 10, or 20 mg/kg. Five minutes postdose, ouabain octahydrate (75 µg/mL) was infused (0.1 mL/min) until an arrhythmia & fibrillation threshold level was achieved. No biologically significant differences in arrhythmia & fibrillation threshold levels were noted between IL-11 & control pigs.

#### **30. Potential Effects of a Test Article in the Anesthetized Ouabain-Intoxicated Guinea Pig**

Guinea pigs were SC injected with IL-11 at 0, 0.1, 1.0, or 10 mg/kg/day for 7 days, followed (24 hours later) by intubation of the jugular vein, carotid artery, & trachea. Ouabain octahydrate (75 µg/mL) was infused (0.1 mL/min) until an arrhythmia & fibrillation threshold level was achieved. No biologically significant differences in arrhythmia & fibrillation threshold levels were noted between IL-11 & control pigs. Clinical pathology changes consisted of what has been described for IL-11 administration: ↑ WBCs, PLTs, & fibrinogen and ↓ albumin & red cell indices.

#### **31. Hemodynamic Effects of Acute IV Administration of IL-11 in New Zealand White Rabbits**

Anesthetized ♀ rabbits were IV injected with IL-11 at 30, 100, & 300 µg/kg [over 2 minutes], with 30 minutes between doses. No significant hemodynamic changes [aortic pressure, peak left ventricular pressure, left ventricular dP/dt max, heart rate] were noted for IL-11 & control animals.

**32. The Cardiovascular Response of IL-11 Treated Beagle Dogs to IV Infusion of Histamine Dihydrochloride**

Beagle dogs were SC injected with vehicle or IL-11 (50, 88, or 136  $\mu\text{g/kg}$ ) over 3 cycles of 12/14 days, with 17 days in between. After catheterization, each dog was IV challenged with 100  $\mu\text{g/kg/min}$  histamine (0.1 mL/kg) for 5 mins, followed by a 10-min rest interval, followed by a second histamine challenge. Mean aortic pressure, ventricular pressure, & heart rate were not affected (consistent with studies #27-31) by IL-11 dosing. Right ventricular pressure decreased in the IL-11 dogs upon the second challenge - possibly due to an inhibitory effect on the histamine-mediated pulmonary venoconstriction. The arterial response was not affected.

**36. Interaction of rhuIL-11 with Cytotoxic Cancer Therapies In Vitro & In Vivo Against Human HT-29 Colon Carcinoma**

Athymic nude mice were implanted with the human colon carcinoma line, HT-29. On days 0-21, IL-11 (125  $\mu\text{g/kg}$ , bid) was SC injected, followed by 5 FU (days 7-11) or fractionated radiation for some mice. Tumor growth delay was similar for 5FU alone (10.9 days) & 5FU/IL-11 (9.7 days). Tumor growth delay produced by fractionated radiation was 13.5 days at 400 rads alone & 23.4 days with IL-11/400 rads.

IL-11 did not protect HT-29 cells grown either as a monolayer in culture, as a solid nodule (SC), or as a solid nodule in the cecum, from the cytotoxic effects of 5FU or radiation. There was no difference in the bone marrow granulocyte:macrophage progenitor cells after treatment with 5FU in mice with & without IL-11 pretreatment.

**37. Interaction of rhuIL-11 with Cytotoxic Cancer Therapies In Vitro & In Vivo Against Murine EMT-6 Mammary Carcinoma**

Balb/c mice were implanted with EMT-6 cells. On days 4-8, IL-11 (125  $\mu\text{g/kg}$ , bid) was SC injected, followed by chemotherapy on day 8 for some mice, followed by kill on day 9. Pretreatment with IL-11 did not alter the response of the tumor cells or the bone marrow granulocyte:macrophage progenitor cells to chemotherapy treatment. The addition of IL-11 resulted in improved survival, improved WBC recovery, & increased tumor growth delay.

**Comment:**

● The Investigator Brochure states that unlike IL-6, IL-11 does not stimulate human myeloma cell in vitro. However, the brochure also notes that IL-11 may indirectly act on B cells, resulting in Ab production via induction of IL-6. The possibility of stimulation of myeloma cells via this IL-6 induction pathway exists.

**38. Alteration in the Frequency, Severity, & Duration of Chemotherapy-Induced Mucositis in Hamsters by IL-11**

Mucositis was induced in male Golden Syrian hamsters by IP injection of 60 mg/kg of 5FU on days 0 and 2, followed by superficial mucosal irritation on day 4. IL-11 was SC injected (3, 10, 30, or 100 µg/day, bid) from days 0-14. A dose-dependent decrease in the severity, frequency, & duration of mucositis occurred. Survival was higher & less weight loss occurred.

**39. Dose Response & Dosing Schedule Studies of rhuIL-11 in HLA-B27 Transgenic Rats**

HLA-B27 transgenic Fischer rats [expressing HLA-B27 & β2-microglobulin genes] exhibit lesions of the GI tract, the joints, gonads, skin - similar to spondylarthropathies in humans & inflammatory bowel disease (100% of the rats): Rats were SC injected with 1000 µg/kg IL-11 [lyophilized, 20 mM histidine, 0.3 M glycine], 5 days/week for 4 weeks. Clinical signs of inflammatory bowel disease (diarrhea, etc.) disappeared & PLTs increased ~26%. Colonic mucosal surface lesions were reduced. In another experiment, IL-11 (10-1000 µg/kg) was injected via various schedules - ranging from 2 days/week x 4 weeks to 5 days/week x 7 weeks. The data showed that shorter weekly schedules of IL-11 were as effective as the longer schedules.

**40. Dose Response & Scheduling Studies of rhuIL-11 in Acetic Acid-Induced Colonic Injury in SD Rats**

Intrarectal instillation of 10% acetic acid, followed by SC injection of IL-11 [lyophilized, 20 mM histidine, 0.3 M glycine] at 10 or 100 µg/kg/day (days 0-5 or 0-14), resulted in significant reduction of gross & microscopic colonic lesions [ulceration, edema, hemorrhage, depletion of goblet cells, infiltration of WBCs], with no difference in the dosing schedules.

**PK/ADME Studies****List of Studies:**

Note that the dates presented with each study are the dates the report was issued, not the date of study completion. The list of studies are numbered in the order in which they appear in the submission.

1. 13-Week SC Dose Toxicokinetic Study of YM294 in Rats; study #KI94085; performed at INA Research, Inc., Japan (per GLP); lot #WN2944A (lyophilized - 0.3 M Glycine/0.01 M Sodium Phosphate); 7/96

2. PK and Bioavailability of rhIL-11 in the Sprague-Dawley Rat; study #PB-012-92; performed at GI (non GLP); lot #NW2570120 (liquid - 0.3 M Glycine/20 mM Histidine); 9/92
3. Toxicokinetics of rhIL-11 in the Cynomolgus Monkey after IV Administration; study #P96084-14/53238; performed at BioResearch & GI (non GLP); lot #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 10/96
4. An Acute PK Study of IL-11 Administered by an IV Bolus Injection and SC Bolus Injection in the Cynomolgus Monkey; study #PS-018-94/53765; performed at BioResearch (non GLP); lot #RB2455-045 (liquid - 0.3 M Glycine/20 mM L-Histidine) & #RB2455-046 (liquid - 0.3 M Glycine/10 mM Sodium Phosphate); 10/96
5. PK of rhIL-11 in Functionally Nephrectomized Sprague Dawley Rats; study #P96125-14; performed at GI (non GLP); lot #0024K01 (lyophilized - 0.3 M Glycine/10 mM Sodium Phosphate); 10/96
6. Biodistribution of rhIL-11 in Normal Sprague Dawley Rats; study #P96124-14; performed at GI (non GLP); lot #0024K01 (lyophilized - 0.3 M Glycine/10 mM Sodium Phosphate); 11/96
7. In Vitro Evaluation of rhIL-11 as a Modulator of Microsomal Cytochrome P450 Expression in Rat and Human Hepatocytes; study #XT 020196/P96110-14; performed at XenoTech L.L.C. (non GLP); lot #TC 0250 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 10/96
8. Evaluation of rhIL-11 as a Suppressor of Liver Microsomal Cytochrome P450 in Rats; study #XT 011196; performed at XenoTech L.L.C. (non GLP); lot #TC 0250 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 9/96

#### PK/ADME Studies

1. 13-Week SC Dose Toxicokinetic Study of YM294 in Rats  
Species: Crj:CD(SD) rats (5/sex/group)  
Formulation: lyophilized  
Dose Level: 0 (vehicle), 1, 10, 100, 1000 µg/kg/day [1 mL/kg]  
Route/Duration: SC/13 weeks

Apparently the lab performed a parallel study in which a small number of rats were dosed with IL-11 for 13 weeks, followed by sample collection for PK. Blood was collected on day 1 and weeks 4 & 13 via the jugular vein of anesthetized rats.

**Findings:** The samples were analyzed by GI. The majority of samples from the 1 µg/kg grp were below the limit of detection (0.2 ng/mL).

**2. PK and Bioavailability of rhIL-11 in the Sprague-Dawley Rat**

**Species:** Crl:CD(SD) ♂ rats (3-4/grp)

**Formulation:** liquid

**Dose Level:** 0 (vehicle), 1, 10, 100, 1000 µg/kg

**Route/Duration:** SC or IV

**Exp. A** - Rats were IV injected with <sup>125</sup>I-IL-11 at 1, 10, 100, or 1000 µg/kg, followed by blood collection (intracardiac puncture) up to 360 mins postdose. Urine & feces were also collected. Total and TCA-precipitable counts were determined.

**Exp. B** - Rats were SC injected with IL-11 at 1 mg/kg/day for 14 days, followed by blood collection (intracardiac puncture).

**Exp. C** - Rats were SC injected with <sup>125</sup>I-IL-11 at 100 µg/kg, followed by blood collection (intracardiac puncture) up to 4440 mins postdose.

The linear trapezoidal rule was used to estimate bioavailability for the SC data. Data were evaluated using PCNONLIN.

**Findings:**

**Exp. A** - A biexponential curve was displayed, with half-lives of 1 min [distributional] and 89 mins. Increased doses resulted in decreased total clearance - from 20-to 13 mL/min/kg. The radiolabel (81%) was recovered in the urine (98% nonprotein bound), with 19% found in the feces.

**Exp. B** - The  $T_{max}$  = 598 mins [0.7% of the injected dose]  
Bioavailability = ~81%

**Exp. C** - A 2.2-fold increase in total clearance occurred.

**3. Toxicokinetics of rhIL-11 in the Cynomolgus Monkey after IV Administration**

**Species:** cyno monkeys (3/sex/group)

**Formulation:** lyophilized

**Dose Level:** 0 (saline & vehicle), 10, 100, 1000, 10,000 µg/kg

**Route/Duration:** IV bolus/single dose

Part of toxicity study #3 (under toxicity study listings)

**Methods:** Blood was collected up to day 7. Sera were analyzed via ELISA for IL-11 levels; the limit of detection was 2 ng/mL. The PK parameters were determined by non-compartmental methods.

Findings: The mean PK data are presented below:

Table 2 Maximum Observed Concentration (C<sub>max</sub>), Initial Volume of Distribution (V<sub>i</sub>), and Steady State Volume (V<sub>ss</sub>), in all Dosing Groups

| rhIL-11 Dose<br>(n = 6 for each group) | C <sub>max</sub><br>(ng/mL) | V <sub>i</sub><br>(mL/kg) | V <sub>ss</sub><br>(mL/kg) |
|----------------------------------------|-----------------------------|---------------------------|----------------------------|
| 10 µg/kg                               | 125 ± 23                    | 82 ± 16                   | ND                         |
| 100 µg/kg                              | 2,652 ± 704                 | 40 ± 11                   | 53 ± 13                    |
| 1,000 µg/kg                            | 21,313 ± 2,334              | 47 ± 6                    | 80 ± 13                    |
| 10,000 µg/kg                           | 333,395 ± 107,045           | 33 ± 13                   | 62 ± 35                    |

ND = not determined

Table 3 Pharmacokinetic Parameter Estimates of AUC<sub>0-6</sub>, AUC<sub>0-24</sub>, Clearance (CL), and Mean Residence Time (MRT) for All Dosing Groups

| rhIL-11 Dose | Time of Last C <sub>s</sub><br>(hours) | AUC <sub>0-6</sub><br>(ng x hours/mL) | AUC <sub>0-24</sub><br>(ng x hours/mL) | CL<br>(mL/hour/kg) | MRT<br>(hours) |
|--------------|----------------------------------------|---------------------------------------|----------------------------------------|--------------------|----------------|
| 10 µg/kg     | 0.75 (n = 6)                           | 26 ± 9                                | ND                                     | ND                 | ND             |
| 100 µg/kg    | 1.5 (n = 1)<br>3 (n = 4)<br>6 (n = 1)  | 581 ± 116                             | 585 ± 116                              | 177 ± 36           | 0.30 ± 0.07    |
| 1,000 µg/kg  | 6 (n = 2)<br>24 (n = 4)                | 8,788 ± 1,649                         | 8,817 ± 1,642                          | 117 ± 22           | 0.72 ± 0.25    |
| 10,000 µg/kg | 48 (n = 6)                             | 231,518 ± 108,490                     | 231,558 ± 108,499                      | 51 ± 21            | 1.17 ± 0.24    |

ND = not determined

T<sub>max</sub> was at 2 mins in 23/24 animals and at 15 mins in 1/24 animals. No measurable IL-11 was detected after 45 mins at 10 µg/kg; after 6 hrs at 100 µg/kg; and after 24 hrs at 1000 µg/kg.

Gender did not alter the PK profile.

Note that based on analysis of the dosing solutions (via bioassay) the activities of the ≥100 µg/kg doses were 2-6-fold higher than the targeted dose, thus dose proportionality was not assessed by the sponsor.

#### 4. A PK Study of IL-11 Administered by an IV Bolus Injection and SC Bolus Injection in the Cynomolgus Monkey

Species: cyno monkeys (3/group)

Formulation: liquid

Dose Level: 100 µg/kg/dose

Route/Duration: SC & IV bolus/single dose

Methods: One group was IV injected with the phosphate/glycine formulation (#A), another was SC injected with the phosphate/glycine formulation (#B), and a third group was SC injected with the histidine/glycine formulation (#C). Each group received all treatments in three weeks (crossover design). Sera were analyzed via ELISA for IL-11 levels; the limit of quantitation was 2 ng/mL. The PK parameters were determined by non-compartmental methods.

Findings: The mean PK data are presented below:

| Parameter/Group               | A                  | B                 | C                 |
|-------------------------------|--------------------|-------------------|-------------------|
| $C_{max}$ (ng/mL)             | $1290.8 \pm 629.6$ | $30.2 \pm 9.8$    | $38.9 \pm 13.9$   |
| $T_{max}$ (min)               | $2.7 \pm 1.3$      | $163.3 \pm 45.3$  | $173.3 \pm 36.1$  |
| $K_{el}$ (min <sup>-1</sup> ) | $0.023 \pm 0.0.3$  | $0.004 \pm 0.001$ | $0.004 \pm 0.001$ |
| $T_{1/2}$ (min)               | $217.9 \pm 266.7$  | $179.1 \pm 30.5$  | $182.7 \pm 57.3$  |
| $MRT_{0-180}$ (min)           | $59.04 \pm 93.4$   | $293.1 \pm 27.2$  | $300.5 \pm 43.8$  |
| $MRT_{\infty}$ (min)          | $123.0 \pm 230.3$  | $351.4 \pm 36.0$  | $356.6 \pm 33.4$  |
| CL (mL/min/kg)                | $5.3 \pm 1.4$      | ND                | ND                |

The absolute bioavailability for the Na phosphate/glycine formulation was  $61\% \pm 20\%$ .

[Per the sponsor] Bioequivalence was established if the 90% confidence interval for the mean PK parameter estimate ( $C_{max}$ ,  $\log_{10}AUC$ , and  $\log_{10}C_{max}$ ) ratio between the two formulations was  $\pm 20\%$ . As is seen in the following table, the 90% confidence interval was not within the 0.80 and 1.20 range that was set. However, upon log transformation of AUC and  $C_{max}$ , the confidence interval fell within the set range.

| Parameter                 | Mean Ratio | 90% Confidence Interval |
|---------------------------|------------|-------------------------|
| $C_{max}$                 | 0.84       | 0.738, 0.948            |
| $AUC_{0-\infty}$          | 0.80       | 0.756, 0.843            |
| $\log_{10}C_{max}$        | 0.95       | 0.915, 0.985            |
| $\log_{10}AUC_{0-\infty}$ | 0.98       | 0.971, 0.982            |

##### 5. PK of rhIL-11 in Functionally Nephrectomized Sprague Dawley Rats

**Methods:** Nephrectomized rats with catheters placed in the jugular vein, along with sham controls, were singly IV injected with 200  $\mu$ g/kg of IL-11, followed by blood collection to 180 minutes postdose. Sera samples were measured using a sandwich ELISA with a lower limit of quantitation of 1.88 ng/mL. The PK parameters were determined by non-compartmental methods.

Findings: The mean PK data are presented below:

Table 2 Pharmacokinetic Parameter Estimates of Serum IL-11 Determined in Sham and Functionally Nephrectomized Rats

|                                    | Sham operated | Functionally Nephrectomized |
|------------------------------------|---------------|-----------------------------|
| C <sub>max</sub> (ng/mL)           | 5285 ± 2183   | 7721 ± 2015                 |
| T <sub>max</sub> (minutes)         | 73 ± 48       | 183 ± 43                    |
| AUC <sub>0-180</sub> (ng × min/mL) | 17710 ± 9238  | 48781 ± 19281               |
| MRT (minutes)                      | 5.0 ± 2.7     | 12.7 ± 8.1                  |

T<sub>max</sub> was 1.1 min (±1.2 min) and 0.9 min (±0.7 min) for nephrectomized and sham rats, respectively. By 180 mins, 7/8 sham rats had IL-11 levels below detectable limits, while the IL-11 levels in the nephrectomized rats was ~4-fold higher than this lower limit. The mean C<sub>max</sub> increased by 68% in the nephrectomized rats compared to sham rats.

#### 6. Biodistribution of rhIL-11 in Normal Sprague Dawley Rats

**Methods:** Normal rats with catheters placed in the jugular vein were singly IV injected with <sup>111</sup>In-IL-11 (200 µg/kg), followed by blood & tissue collection to 90 minutes postdose. Sera samples were measured using a sandwich ELISA [lower limit of quantitation of 1.88 ng/mL] and by gamma counting.

**Findings:** About 89% & 5% of the radiolabel distributed to the kidneys and liver, respectively, with <3% found in the spleen and lung. The ratio of ng equivalent of <sup>111</sup>In-IL-11 in the kidney to the ng equivalent of <sup>111</sup>In-IL-11 in the sera increased by 180-fold by 90 mins. The label cleared rapidly from the sera as it accumulated in the kidney.

#### Comment:

● Based on the data in the submission, an earlier PK study was performed (at GI, in 9/92, study #PB-013-92) using normal SD rats and <sup>125</sup>I-IL-11. The distribution to the kidneys & liver was found to be 2.4% & 31.3%, respectively. Based on other studies using <sup>111</sup>In label, the sponsor suspected that the <sup>125</sup>I label resulted in erroneous data due to labeling techniques that may have altered the distribution profile of IL-11, or other technical factors such as tissue sample collection.

## 7. In Vitro Evaluation of rhIL-11 as a Modulator of Microsomal Cytochrome P450 Expression in Rat and Human Hepatocytes

**Methods:** The ability of IL-11 to induce or attenuate the P450 enzyme-inducing effects of  $\beta$ -naphthoflavone (CYP1A inducer), phenobarbital (CYP2B inducer), dexamethasone & rifampin (CYP3A inducers), and clofibric acid (CYP4A inducer) in primary cultures of rat and human hepatocytes, was determined. P450 enzyme specific assays and western immunoblotting techniques were used.

IL-11 [2-50 ng/mL] suppressed the induction of cytochrome P450 in rat hepatocytes as follows: CYP2B > CYP3A > CYP4A  $\geq$  CYP1A. IL-11 was also more effective than LPS (34 ng/mL) at suppressing the induction of P450 enzymes. In human hepatocytes, LPS was more effective than IL-11 at suppressing induction of P450 enzymes [CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C10, CYP3A4].

### Comment:

- Infection and inflammation can impair the metabolism of drugs due to a decrease in the expression of one or more cytochrome P450 enzymes in the liver microsomes. Cytokines help to mediate the suppression of P450 expression.

## 8. Evaluation of rhIL-11 as a Suppressor of Liver Microsomal Cytochrome P450 in Rats

**Methods:** Male SD rats were IP injected with 50, 200, or 1000  $\mu$ g/kg of IL-11, followed by kill at 24 hrs, to prepare the liver microsomes. Positive controls received LPS and negative controls received saline. An IL-11 dose of 1000  $\mu$ g/kg resulted in a 32% decrease in the O-dealkylation of 7-ethoxyresorufin, 39% decrease of O-dealkylation of 7-ethoxycoumarin, 39% decrease in the hydroxylation of para-nitrophenol, and 32% decrease in the 11-hydroxylation of lauric acid - suggestive of decreased levels of CYP1A2 & CYP2E1. The 1000  $\mu$ g/kg dose also resulted in a 35-36% drop in testosterone 2 $\alpha$  and 16 $\alpha$ -hydroxylase activity - markers of CYP2C11 - and a 38-39% drop in testosterone 2 $\beta$  and 6 $\beta$ -hydroxylase activity - markers of CYP3A2. The decrease in P450 enzyme activities appeared to be dose-related. The changes noted reflect possible decreases in the oxidative metabolism of various drugs with IL-11.

Note that LPS was 2-fold more effective than IL-11 (at 1000  $\mu$ g/kg) in causing a decrease in P450 enzyme activity.

Preclinical Toxicology Studies

## List of Studies:

Note that the dates presented with each study are the dates the report was issued, not the date of study completion.  
The list of studies are numbered in the order in which they appear in the submission.

1. Single IV Dose Toxicity Study of YM294; study #393407; performed at Yamanouchi Pharmaceutical (non GLP); lot #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 7/93
2. Single SC Dose Toxicity Study of YM294 in Rats; study #393408; performed at Yamanouchi Pharmaceutical (non GLP); lot #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 7/93
3. A Single Dose IV Injection Toxicity Study of IL-11 in the Cynomolgus Monkey, with a 14-Day Observation Period; study #53238; performed at BioResearch (per GLP); lot #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 11/94
4. Dose Finding Study for the Repeated Dose Toxicity Study of YM294 [rhIL-11] Administered as IV Infusion in Rats; study #392311; performed at Yamanouchi Pharmaceutical (non GLP); lot #TOX012 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 11/92
5. 4-Week Repeated IV Dose Toxicity Study of YM294 [rhIL-11] in Rats; study #KI92127; performed at Yamanouchi Pharmaceutical (per GLP); lot #TOX012 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 4/93
6. A 28-Day Toxicity Study of IL-11, Administered SC in the Albino Rat with a 28-Day Recovery Period; study #53181; performed at BioResearch (per GLP); lot #NW2570103 (liquid - 0.3 M Glycine/20 mM L-Histidine); 12/92
7. A 28-Day Toxicity Study of Lyophilized IL-11, Administered SC in the Albino Rat, Followed by a 28-Day Recovery Period; study #53526; performed at BioResearch (per GLP); lot #TOX012 (lyophilized - 0.3 M Glycine/20 mM L-Histidine), #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 11/94
8. 13-Week SC Dose Toxicity Study of YM294 [rhIL-11] in Rats with an 8-Week Recovery Period; study #KI94033; performed at INA Research, Inc., Japan (per GLP); lot #WN2944A (lyophilized - 0.3 M Glycine/10 mM Sodium Phosphate); 7/96

9. A 14-Day SC Toxicity Study of IL-11 in the Cynomolgus Monkey; study #52896; performed at BioResearch (per GLP); lot #TOX012 (lyophilized - 0.3 M Glycine/20 mM L-Histidine), #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 3/92

10. An Interaction Toxicity Study of IL-11 in Combination with G-CSF or GM-CSF in Cynomolgus Monkeys Followed by a 14-Day Recovery Period; study #53423; performed at BioResearch (per GLP); lot #TOX012 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); GM-CSF from Schering (lot #FMR91647D02); G-CSF from Amgen (lot #688H2); 10/96

11. A 28-Day Toxicity Study of IL-11 Administered SC in the Cynomolgus Monkey, with a 28-Day Recovery Period; study #53852; performed at BioResearch (per GLP); lot #RB2455-064 (liquid - 0.3 M Glycine/10 mM Sodium Phosphate); 8/94

12. A 28-Day Toxicity Study of IL-11 Administered SC in the Cynomolgus Monkey, with a 28-Day Recovery Period; study #53058; performed at BioResearch (per GLP); lot #NW2570103 (liquid - 0.3 M Glycine/20 mM Histidine); 12/92

13. YM294: Toxicity to Cynomolgus Monkeys by Repeated SC Administration for 4 Weeks; study #YMI 91/931340; performed at Huntingdon Life Sciences, Ltd. (per GLP); lot #TOX012 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 11/95

14. YM294 [rhIL-11]: Toxicity to Cynomolgus Monkeys by Repeated IV Infusion Administration for 4 Weeks Followed by a 4-Week Recovery Period; study #YMI 86/932353; performed at Huntingdon Life Sciences, Ltd. (per GLP); lot #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 11/95

15. YM294: Toxicity to Cynomolgus Monkeys by Daily SC Administration for Up to 13 Weeks Followed by a Recovery Period; study #YMI 145/951558; performed at Huntingdon Life Sciences, Ltd. (per GLP); lot #WN2944A (lyophilized - 0.3 M Glycine/10 mM Sodium Phosphate); 7/96

**Comment:**

- Many of the toxicology study reports are still in draft form & are not signed.

### Acute Toxicity Studies

**Note:** The final IL-11 formulation contains a small amount of a fusion protein contaminant (thioredoxin), which can affect immune function. The clinical formulation contains ~0.1% (10 ng/10 µg IL-11) of this contaminant. The TOX-015 lot contains a high amount - 5.48 ng/10 µg - of the thioredoxin moiety, thus was used in various preclinical studies for evaluation of additional toxicities.

#### 1. Single IV Dose Toxicity Study of YM294

Species: Crj:CD(SD) rats (5/sex)

Formulation: lyophilized

Dose Level: 10 mg/kg

Route/Duration: IV/single dose + 2 weeks observation

**Findings:** Transient BW loss; transient hypoactivity & auricular redness

#### 2. Single SC Dose Toxicity Study of YM294 in Rats

Species: Crj:CD(SD) rats (5/sex)

Formulation: lyophilized

Dose Level: 10 mg/kg

Route/Duration: SC/single dose + 2 weeks observation

**Findings:** Transient hypoactivity & auricular redness

#### 3. A Single Dose IV Injection Toxicity Study of IL-11 in the Cynomolgus Monkey, with a 14-Day Observation Period

Species: cyno monkeys (3/sex/grp)

Formulation: lyophilized

Dose Level: 0 (saline & vehicle), 10, 100, 1000, 10,000 µg/kg

Route/Duration: IV/single dose + 2 weeks observation

**Methods:** Clinical signs, BWs, appetite, clinical pathology (daily for days 1-7 & on day 14), ophthalmoscopy, sera IL-11 levels, and gross & microscopic pathology were performed.

**Findings:** No abnormalities in clinical signs, BWs, appetite, ophthalmoscopy, body temps, & gross pathology

↑ WBCs, neutrophils - Rx ♂s & 10,000 µg/kg ♀s - day 1 (postdose)

↑ PLTs - 10,000 µg/kg ♂s - days 6, 7, 14

- 1000 µg/kg ♂s - days 6, 7

- 10 µg/kg ♂s - day 14

↑ fibrinogen - ≥100 µg/kg ♂s - days 2-7

- Rx ♀s ♂s - days 2-7 (sporadic, dose-related)

↓ red cell mass - all grps (due to phlebotomy) - slightly more in IL-11 grps

↑ globulin; ↓ A/G -  $\geq 1000 \mu\text{g/kg}$  - day 14

↓ albumin, alpha & gamma globulin -  $10,000 \mu\text{g/kg}$  ♂s - day 14

↑ AlkP (liver derived - absolute change occurred here); ↓ AlkP (bone derived) -  $10,000 \mu\text{g/kg}$

↑ femoral/sternal BM hematopoiesis -  $10,000 \mu\text{g/kg}$

↑ extramedullary hematopoiesis - liver -  $1000 \mu\text{g/kg}$  (1/6) &  $10,000 \mu\text{g/kg}$  (6/6)

The NOAEL =  $10,000 \mu\text{g/kg/day}$

#### Multidose Toxicity Studies

#### 4. Dose Finding Study for the Repeated Dose Toxicity Study of YM294 [rhIL-11] Administered as IV Infusion in Rats

Species: SD rats (3 ♂s/grp)

Formulation: lyophilized

Dose Level: 0,  $870 \mu\text{g/kg/day}$

Route/Duration: SC injection or 1-hr IV infusion/7 days + 8-day observation period

Blood collected on day 2 & postdose days 4 & 8

#### Findings:

↑ PLTs - both routes - postdose day 4

#### 9. A 14-Day SC Toxicity Study of IL-11 in the Cynomolgus Monkey

Species: cyno monkeys (6 ♂s/grp)

Formulation: lyophilized

Dose Level: 30, 100,  $300 \mu\text{g/kg}$  - no controls were run

Route/Duration: SC/14 days + kills on days 7 & 15

Methods: Clinical signs, BWs, body temperatures, clinical pathology (baseline & days 8 & 15), PLT function (aggregation, thromboxane B<sub>2</sub>, platelet factor 4), and gross and histology were performed.

Findings: [relative to baseline, as no controls were run]

No abnormalities in clinical signs, BWs, appetite, PLT aggregation, total bleeding times, M/E ratios, organ weights, or gross pathology findings

↑ body temps - Rx grps - throughout the study

↓ red cell mass; ↑ PLTs - Rx grps - days 7, 15  
Giant PLT cells present - Rx grps - days 7, 15

↑ AlkP; ↓ albumin, total protein, A/G ratio, chloride, calcium, phosphorus - Rx grps - days 7, 15

Femur - subperiosteal hyperostosis - 3/3 (100 µg/kg) & 2/3 (300 µg/kg); joint capsular/tendon insertion fibrosis - 1/3 (100 µg/kg) & 3/3 (300 µg/kg) - day 15

Electron microscope work indicated a possible immaturity of the megakaryocytes in the BM at 300 µg/kg [↓ granules in the PLT field of the cytoplasmic intermediate zone of megakaryocytes]

The NOAEL was 30 µg/kg

#### 5. 4-Week Repeated IV Dose Toxicity Study of YM294 [rhIL-11] in Rats

Species: Crj:CD(SD) rats (10-15/sex/grp)

Formulation: lyophilized

Dose Level: 0, 1, 10, 100, 1000 µg/kg/day

Route/Duration: 1-hr IV infusion/4 weeks + 4-week recovery

Methods: Clinical signs, BWs, food & water consumption, ophthalmoscopy, clinical pathology (4 & 8 weeks), anti-IL-11 Abs, organ weights, and gross & histopathology (1000 µg/kg grp & lesions only) were performed.

#### Findings:

Tail bent (injection site) - 1000 µg/kg - beginning on day 23 m [due to decreased mobility of tail from ↑ connective tissues around the muscle bundle]

↓ BW gain, food consumption - 1000 µg/kg - beginning in week 2 - recovery

Insufficient mydriasis [following instillation of mydriatic agent] - 1000 µg/kg - week 4

↑ PLTs - ≥100 µg/kg - week 4 - present at week 8 in 1000 µg/kg ♂s

↓ APTT - 1000 µg/kg ♂s - week 4 - recovery

↑ monocytes - 1000 µg/kg ♀s - week 4 - recovery

↓ albumin; ↑ α & β globulin, A/G ratio - ≥100 µg/kg - week 4 - recovery

↑ liver, kidney, adrenal weights - ≥100 µg/kg - week 4

↑ spleen weight - ≥10 µg/kg - week 4 - recovery

↓ seminal vesicle weight - 1000 µg/kg - week 4 - recovery

↓ peritoneal adipose tissue; seminal vesicle atrophy - 1000 µg/kg - recovery



All clinical pathology parameters returned to baseline.

↑ spleen weight - 1000 µg/kg - days 8, 15, 29, 57 (♀s)  
                  - 55 µg/kg - days 15/29  
↑ liver weight - 1000 µg/kg - days 15, 57 (♀s) & 15, 29 (♀s)

Enlarged spleen and lymph nodes - 55, 1000 µg/kg - days 8, 15, 29; 1000 µg/kg - day 57

Histo - The gross findings correlated with slight to moderate lymphoid hyperplasia - still present after recovery at 1000 µg/kg

The NOAEL = 1000 µg/kg/day

**Comments:**

- The increased rate of platelet aggregation was most likely due to the increased number of platelets.
- The increased liver weight, alterations in serum protein profile, and AlkP changes suggest the liver as a potential target organ.
- Increased AlkP levels can be indicative of biliary stasis. However, increased liver weights and alterations in liver enzymes & AlkP suggest the liver as a target organ.

**7. A 28-Day Toxicity Study of Lyophilized IL-11, Administered SC in the Albino Rat, Followed by a 28-Day Recovery Period**

**Species:** Crl:CD(SD)BR rats (20-40/sex/grp)

**Formulation:** lyophilized

**Dose Level:** 0 (saline & vehicle), 1, 10, 30, 100, 300, 1000 µg/kg/day [1 mL/kg]

**Route/Duration:** SC/28 days + 28-day recovery [kills on days 8, 15, 29, 57 for 1000 µg/kg & on days 15, 29 for 1-300 µg/kg]

**Note:** Analysis of the activity of the dosing solutions (via bioassay) revealed that the actual dose of 1 µg/kg may have been as much as 50-90% lower than the targeted dose [i.e., 0.1 µg/kg]; and the actual dose of 10 µg/kg may have been up to 40-60% lower than the targeted dose [i.e., 4 µg/kg]. However, the 1000 µg/kg dose may have been as much as 20-70% **higher** than the targeted dose [i.e., 1700 µg/kg].

This information was amended to the study report in 10/96, but was not submitted to the IND.

**Methods:** Clinical signs, body weights, food consumption, clinical pathology, ophthalmoscopy, organ weights, and gross & microscopic pathology were evaluated.

**Findings:**

Deaths (day 14) - due to blood sampling procedures - two 4 µg/kg and one 30 µg/kg

Hypoactivity; piloerection; redness/edema of extremities; dehydration; and emaciation - ≥100 µg/kg - dose-related

**Note** that dehydration, emaciation, and hypoactivity were predominantly in 1700 µg/kg group [♂s appeared more effected than ♀s]

**Recovery interval** - some dehydration and emaciation - 1700 µg/kg

↓ BW gains - 1700 µg/kg ♂s (22-62% lower than control); with some

↓ BWs for recovery 1700 µg/kg ♀s

↓ food consumption - 1700 µg/kg

↑ neutrophils, lymphocytes, monocytes - 1700 µg/kg - recovery

↓ red cell mass - ≥100 µg/kg - days 7, 14, 28 - recovery

↑ PLTs - ≥30 µg/kg - days 7, 14, 28 - recovery

↑ BUN, phosphorus, globulin, total protein, potassium; ↓ glucose, albumin, A/G - 1700 µg/kg - days 8, 15, 29 - trend toward recovery

↑ BUN, phosphorus, globulin, potassium; ↓ glucose, albumin, A/G - 100, 300 µg/kg - days 15, 29 - recovery

↑ beta/gamma globulin - ≥30 µg/kg - days 8/15/29

↓ AlkP (bone derived); ↑ AlkP (liver derived) - ≥30 µg/kg - days 8/15/29 - dose-related incidence

↑ adrenal weights - ≥300 µg/kg - recovery

↑ spleen, liver weights - ≥30 µg/kg - no recovery at 1700 µg/kg

Injection sites - dark foci/areas - ≥300 µg/kg

↑ mixed cell infiltration at injection sites - 1700 µg/kg - all kills

↑ splenic lymphoid hyperplasia - ≥300 µg/kg - days 15, 29

Thickening of femoral & tibial growth plates (diffuse thickening of cartilage) - ≥100 µg/kg - days 8, 15, 29 - dose-related in onset & severity - trend toward recovery

1700 µg/kg:

Day 29 - the lesions were of greater severity compared to day 15  
6/10 ♂s showed less severe lesions on day 57

**The NOAEL = 30 µg/kg/day**

**Comment:**

● Increases in AlkP can occur due to the action of the osteoblastic cells or from hepatobiliary obstruction.

10. An Interaction Toxicity Study of IL-11 in Combination with G-CSF or GM-CSF in Cynomolgus Monkeys Followed by a 14-Day Recovery Period

Species: cyno monkeys (2 or 4/sex/grp)

Formulation: lyophilized

Dose Level: 0, 100  $\mu\text{g/kg/day}$  = IL-11

10  $\mu\text{g/kg/day}$  = GM-CSF (alone or + IL-11)

5  $\mu\text{g/kg/day}$  = G-CSF (alone or + IL-11)

Route/Duration: SC/14 days + 14-day recovery

IV (all GM-CSF grps) - same regimen

Methods: Clinical signs, body weights, appetite, clinical pathology (baseline and days 1, 14, & 28), body temperature, ophthalmoscopy, organ weights, and gross & microscopic pathology were performed.

Findings:

Hematology -

Day 1 - 6 hrs postdose

↑ WBCs, neutrophils, monocytes, lymphocytes - G-CSF & G-CSF + IL-11 grps

↑ monocytes - IL-11 grp

↑ eosinophils - GM-CSF, G-CSF grps

Day 14

↑ WBCs, neutrophils, monocytes, lymphocytes, eosinophils - G-CSF, GM-CSF, G-CSF + IL-11, GM-CSF + IL-11 grps

Anemia (slight); ↑ APTT, PLTs, RBC distribution width - IL-11 grps

↑ polychromasia, poikilocytosis - Rx grps

Day 28 - trend toward recovery

Chemistry -

↓ bilirubin - day 1 (GM-CSF); day 14 (IL-11, IL-11 + G/GM-CSF)

↑ LDH [LD4/5] - day 1 (IL-11, G/GM-CSF)

↑ AlkP - day 14 - Rx grps [↑ liver fraction; ↓/↑ bone fraction]

↓ albumin; ↑ alpha, beta-globulin, triglycerides - Rx grps

Day 28 - trend toward recovery

PK - Day 1 -

AUC (ng.hr/mL) = 162.2  $\pm$  38.8 (IL-11 alone)

197.4  $\pm$  64.9 (IL-11 + GM-CSF)

197.4  $\pm$  44.1 (IL-11 + G-CSF)

C<sub>max</sub> (ng/mL) = 37.9  $\pm$  6.9 (IL-11 alone)

52.0  $\pm$  24.4 (IL-11 + GM-CSF)

36.8  $\pm$  8.3 (IL-11 + G-CSF)

Day 14 =

AUC (ng.hr/mL) = 185.4  $\pm$  58.5 (IL-11 alone)

208.9 +183.4 (IL-11 + GM-CSF)

308.8 +116.8 (IL-11 + G-CSF)

$$C_{\max} \text{ (ng/mL)} = 62.7 \pm 12.6 \text{ (IL-11 alone)}$$

54.2 +56.8 (IL-11 + GM-CSF)

99.3 +39.6 (IL-11 + G-CSF)

↑ liver, kidney, spleen weight - all IL-11 grps - day 15

Extramedullary hematopoiesis - liver/spleen - all Rx grps -  
days 15, 29

↑ hematopoiesis - BM - Rx grps - days 15, 29

↑ leukocytosis - hepatic sinusoids; cell infiltrates - liver, spleen - Rx grps - various kills

**Comment :**

● The day 1 AUC & C<sub>max</sub> values for the IL-11 alone or combination groups were similar. However, the day 14 AUC & C<sub>max</sub> for IL-11 + G-CSF were slightly higher compared to IL-11 alone or IL-11 + GM-CSF. The significance of these data are questionable, as much intra- and interanimal variation existed and the sample size was small (2-4 monkeys per group). Of note is that there was no apparent unexpectd effects on the hematopoietic parameters evaluated nor the toxicology of the various combinations of agents.

11. A 28-Day Toxicity Study of IL-11 Administered SC in the Cynomolgus Monkey, with a 28-Day Recovery Period

**Species:** cyno monkeys (4 or 8/sex/grp)

Formulation: liquid

Dose Level: 0 (saline & vehicle), 1, 100, 300, 1000  $\mu\text{g/kg/day}$

**Route/Duration:** SC (dorso-thoracic region)/28 days + 28-day recovery (high dose only)

**Note:** The toxicology study evaluated the new formulation of IL-11. The old formulation was in a lyophilized, preservative-free form with L-histidine and glycine, with a pH (when reconstituted with sterile water) of 7.0.

**Note:** Analysis of the activity of the dosing solutions (via bioassay) revealed that the actual dose of 1 µg/kg may have been as much as 70-100% lower than the targeted dose [i.e., 0-0.3 µg/kg]. This information was amended to the study report in 10/96, but was not submitted to the IND.

**Methods:** Clinical signs, BWs, appetite, clinical pathology (baseline & days 7, 14, 21, 28, 50, 56 for hematology and baseline & days 28, 56 for chemistry), platelet aggregation,

ophthalmoscopy, organ weights, rectal temperatures, ECGs, sera IL-11 levels, anti-IL-11 Abs, organ weights, and gross & microscopic pathology were evaluated.

**Findings:**

No abnormalities in BWs, appetite, ECGs, rectal temps

Partly closed eyes -  $\geq 300 \mu\text{g/kg}$  - incidence is dose-dependent

Swollen hindlimb(s) -  $\geq 300 \mu\text{g/kg}$

Red hindlimb(s) - vehicle;  $\geq 300 \mu\text{g/kg}$

The signs generally resolved during recovery.

**Ophthalmoscopy** - bilateral swelling and congestion of optic nerve head & peripapillary retina -  $1000 \mu\text{g/kg}$  - resolved. There was no microscopic correlate.

**Clinical Pathology** - (not dose-dependent)

↑ neutrophils -  $\geq 100 \mu\text{g/kg}$  - days 7, 14, 21, 28

↓ lymphocytes -  $\geq 100 \mu\text{g/kg}$  - days 7, 14, 21, 28

↓ red cell mass -  $\geq 100 \mu\text{g/kg}$  - days 7, 14

↑ retics -  $\geq 100 \mu\text{g/kg}$  - days 7, 14

↓ MCV, MCHC, MCH -  $\geq 100 \mu\text{g/kg}$  - days 14, 21, 28

↑ platelets, fibrinogen -  $\geq 100 \mu\text{g/kg}$  - days 7, 14, 21, 28, 36

↑ APTT -  $\geq 100 \mu\text{g/kg}$  - day 28

Platelet aggregation - ↑ rate -  $\geq 100 \mu\text{g/kg}$  - days 3, 28

↑ hypochromasia, polychromasia, codocytes, schizocytes -

$\geq 100 \mu\text{g/kg}$  [reflective of anemia]

Giant platelets -  $\geq 300 \mu\text{g/kg}$  - day 28

↓ M/E ratio -  $\geq 100 \mu\text{g/kg}$  - day 28

↓ albumin, bilirubin, A/G ratio; ↑ AlkP, globulin, potassium, haptoglobin,  $\alpha$  &  $\beta$  globulin, acid  $\alpha$ -1-glycoprotein,  $\alpha$ -1-antitrypsin, apolipoprotein B, properdin factor B -  $\geq 100 \mu\text{g/kg}$  - day 28

↑ BUN, LDH, complement C3 & C4; ↓ ALT, GGT, calcium, protein, glucose -  $300/1000 \mu\text{g/kg}$  - day 28

Generally, all parameters returned to baseline levels during recovery.

↑ liver, spleen, kidney weights -  $\geq 100 \mu\text{g/kg}$  - resolved

Thickening of SC tissue in hindlimb(s) -  $\geq 300 \mu\text{g/kg}$  - day 29 - resolved

**Day 29 Kill -**

↑ hematopoiesis (mild) - bone marrow -  $\geq 100 \mu\text{g/kg}$

**Liver** - slight/mild mixed cell infiltration (sinusoids) -  $1000 \mu\text{g/kg}$

**Injection site(s)** - slight/mild cellulitis -  $\geq 300 \mu\text{g/kg}$

Edema/fibrosis in SC tissue at the hindlimb(s) -  $\geq 300 \mu\text{g/kg}$

**Femur/sternum** - subperiosteal hyperostosis [slight/mild]; joint capsular/tendon insertion fibrosis [slight/mild] -  $\geq 100 \mu\text{g/kg}$

All lesions resolved, except for:

↑ hematopoiesis in the BM & subperiosteal hyperostosis (sternum) in one  $1000 \mu\text{g/kg}$  ♂

Joint capsular/tendon insertion fibrosis in 4/4 ( $1000 \mu\text{g/kg}$ ) monkeys

**Note** that only  $1000 \mu\text{g/kg}$  animals were killed at recovery, so it cannot be assumed that the bone findings in the  $100$  &  $300 \mu\text{g/kg}$  animals resolved.

The NOAEL was  $0-0.3 \mu\text{g/kg/day}$

**Comments:**

- The increased rate of platelet aggregation was most likely due to the increased number of platelets. The changes in blood cell morphology are indicative of anemia, followed by an increase in RBC production and recovery.

- The sera chemistry changes indicate that the liver may be a target organ.

**12. A 28-Day Toxicity Study of IL-11 Administered SC in the Cynomolgus Monkey, with a 28-Day Recovery Period**

**Species:** cyno monkeys (4 or 8/sex/grp)

**Formulation:** liquid

**Dose Level:** 0, 1, 100,  $1000 \mu\text{g/kg/day}$

**Route/Duration:** SC/28 days + 28-day recovery (high-dose only)

**Note:** Analysis of the activity of the dosing solutions (via bioassay) revealed that the actual dose of  $1 \mu\text{g/kg}$  may have been as much as 80-90% lower than the targeted dose [i.e.,  $0.1-0.2 \mu\text{g/kg}$ ]. This information was amended to the study report in 10/96, but was not submitted to the IND.

**Methods:** Clinical signs, BWs, appetite, clinical pathology (baseline and days 7, 14, 21, 28, 36, 43, 50, & 56 - to include PLT aggregation), body temperature, ophthalmoscopy, organ weights, and gross & microscopic pathology were performed.

**Findings:**

Death - one 1000  $\mu\text{g/kg}$   $\delta$  - day 23 -  $\downarrow$  BWs, red cell mass;  $\uparrow$  PLTs (3-4-fold baseline); no microscopic abnormalities

Moribund - one 1  $\mu\text{g/kg}$   $\delta$  - day 29 - multiple pulmonary thrombi eliciting edema due to venous congestion were noted

Hypoactivity - two 1000  $\mu\text{g/kg}$   $\delta$ s - days 20-28 (sporadically) - lasting from 1-30 min. postdose

$\downarrow$  WBC -  $\geq 100$   $\mu\text{g/kg}$   $\delta$ s - day 28 - recovery by day 36

$\downarrow$  lymphocytes - 1000  $\mu\text{g/kg}$   $\delta$ s - recovery by day 36

$\uparrow$  APTT -  $\geq 100$   $\mu\text{g/kg}$  - day 28

$\downarrow$  red cell mass -  $\geq 100$   $\mu\text{g/kg}$  - day 7 (dose-related) - recovery trend by day 28

$\uparrow$  retics - Rx grps - days 28 (dose-related)

$\uparrow$  PLTs -  $\geq 100$   $\mu\text{g/kg}$  - during Rx (2-4-fold baseline) - recovery by day 43

$\uparrow$  RBC distribution width - during Rx -  $\geq 100$   $\mu\text{g/kg}$

$\uparrow$  poly- & hypochromasia, poikilocytosis - day 28 -  $\geq 100$   $\mu\text{g/kg}$  - recovery [reflective of anemia]

$\downarrow$  M/E ratio - days 15, 29 -  $\geq 100$   $\mu\text{g/kg}$  - recovery by day 57

Platelet aggregation - no apparent effect

$\uparrow$   $\alpha$ -1/2 &  $\beta$  globulins -  $\geq 100$   $\mu\text{g/kg}$  - day 28

$\uparrow$  AlkP - 1000  $\mu\text{g/kg}$  - day 28 - all isoenzymes

$\uparrow$  triglycerides -  $\geq 100$   $\mu\text{g/kg}$  - day 28 (dose-related)

$\downarrow$  protein, albumin;  $\uparrow$  globulin -  $\geq 100$   $\mu\text{g/kg}$  - day 28 (dose-related)

All parameters returned to baseline.

$\uparrow$  liver, kidney, spleen weight -  $\geq 100$   $\mu\text{g/kg}$  - day 28; liver & spleen remained  $\uparrow$  at recovery

**Day 29 Kill**

Liver -  $\uparrow$  leukocytosis - sinusoids - Rx grps (dose-related) - noted at recovery

BM -  $\uparrow$  hematopoiesis - Rx grps (dose-related) - noted at recovery

Femur/sternum - subperiosteal hyperostosis [slight/mild]; joint capsular/tendon insertion fibrosis [slight/mild] -  $\geq 100$   $\mu\text{g/kg}$  - noted at recovery

Note that only 1000  $\mu\text{g/kg}$  animals were killed at recovery, so it cannot be assumed that the bone findings in the 100  $\mu\text{g/kg}$  animals resolved.

The NOAEL was 0.1-0.2  $\mu\text{g/kg/day}$

**Comment:**

● The report attributes the death to the numerous phlebotomies - this reviewer does not agree with this explanation. The sponsor offers the explanation that the animal developed viral pericarditis that altered cardiac dynamics and allowed the formation of thrombi in the right ventricle. The primate had high PLT counts.

Note that in study #18 in the preclinical pharmacology section, deaths occurred in IL-11 exposed normal & myelosuppressed beagle dogs from hemorrhagic pneumonia. The lungs contained vascular congestion & hemorrhage. There exists a potential relationship between IL-11-induced thrombocytosis and these hemorrhages.

**13. YM294: Toxicity to Cynomolgus Monkeys by Repeated SC Administration for 4 Weeks**

Species: cyno monkeys (3/sex/grp)

Formulation: lyophilized

Dose Level: 0, 10, 300 µg/kg/day

Route/Duration: SC/28 days

Methods: Clinical signs, BWs, appetite, clinical pathology (baseline and weeks 1, 2, 3, 4 for hematology; baseline and week 4 for chemistry), anti-IL-11 Abs, PK samples, organ weights, and gross & microscopic pathology were performed.

**Findings:**

Hypoactivity, huddled posture, piloerection - Rx ♂s

↑ incidence of aggression - 300 µg/kg ♂s

↓ BW gain, appetite - 300 µg/kg ♂s

↓ HCT, HGB, RBCs - 300 µg/kg ♂s - weeks 1-4

↓ HCT, HGB, RBCs - 10 µg/kg ♀s [slight] - weeks 1-4

↑ retics - 300 µg/kg - weeks 2, 4

↓ MCV - 300 µg/kg - weeks 3, 4

↑ hypochromasia, polychromasia, anisocytosis, rouleaux - 10 µg/kg (both sexes) & 300 µg/kg ♂s (dose-related) - weeks 2, 4

↑ PLTs - Rx grps (dose-related) - weeks 1-4

↑ APTT - Rx ♂s - weeks 2, 4

↓ WBCs (lymphocytes) - Rx ♂s - weeks 2, 4

↓ M/E ratio [megalo-blastic-like development of RBCs; ↑ early normoblasts & intermediate normoblasts] - Rx ♂s - week 4

↓ albumin, A/G ratio; ↑ globulin [α-1, β, γ-globulins] - Rx grps - week 4

↑ AlkP - 300 µg/kg ♂s - week 4  
↓ CPK - Rx grps - week 4  
↑ 5' nucleotidase - 300 µg/kg ♂s - week 4

↑ liver weights - 300 µg/kg ♂s

Thymus - cortical atrophy - 300 µg/kg ♂s  
Liver - hepatocyte enlargement - 300 µg/kg ♂s

The NOAEL was 10 µg/kg/day

**14. YM294 [rhIL-11]: Toxicity to Cynomolgus Monkeys by Repeated IV Infusion Administration for 4 Weeks Followed by a 4-Week Recovery Period**

Species: cyno monkeys (3 or 5/sex/grp)

Formulation: lyophilized

Dose Level: 0, 1, 10, 100, 300 µg/kg/day

Route/Duration: IV (1-hr infusion [animals chaired], 5 mL/kg; rate adjusted for each animal to complete dosing in 1 hr)/28 days + 28-day recovery period (high dose only)

Methods: Clinical signs, BWs, appetite, body temperature, ECGs, clinical pathology (baseline and weeks 2, 4 & recovery weeks 1, 3, & 4), ophthalmoscopy, anti-IL-11 Abs, PK samples, organ weights, and gross & microscopic pathology were performed.

**Findings:**

No abnormalities in BWs, appetite, body temperatures, ophthalmoscopy.

Huddled posture - ≥100 µg/kg - weeks 2-4 - recovery  
Hypoactivity, piloerection, loose feces - 300 µg/kg - weeks 3, 4 - recovery  
Lumps/swellings - injection site - 300 µg/kg - recovery

↓ mean heart rate - ≥100 µg/kg ♂s - recovery

↓ HCT, HGB - ≥10 µg/kg ♂s, 300 µg/kg ♀s - weeks 2, 4

↓ RBC - 300 µg/kg - week 2 - recovery

↑ retics - 300 µg/kg - weeks 2, 4 [slight ↑ retics for individual animals at 10, 100 µg/kg - weeks 2/4]

↑ hypochromasia, anisocytosis, rouleaux - 300 µg/kg - weeks 2, 4 - recovery

↑ PLTs - ≥100 µg/kg - weeks 2, 4 - trend toward recovery

↓ PT - ≥10 µg/kg - week 4 - recovery

↓ WBCs (lymphocytes) - ≥100 µg/kg ♂s - weeks 2, 4 - trend toward recovery

↓ M/E ratio [↑ early normoblasts & intermediate normoblasts] -  
≥100 µg/kg ♂s - week 4

↓ albumin, A/G ratio; ↑ globulin [α-1, β, γ-globulins] -  
≥100 µg/kg - weeks 2, 4 - trend toward recovery

↓ albumin, A/G ratio; ↑ globulin - 10 µg/kg - weeks 2/4 -  
recovery

↑ AlkP - 300 µg/kg ♀s - weeks 2, 4 - recovery

↓ ALT, AST, CPK, LDH - ≥100 µg/kg - weeks 2, 4 - recovery

↓ leucine aminopeptidase - ≥10 µg/kg - weeks 2, 4 - recovery

#### Week 4 Kill

↑ liver weights - ≥10 µg/kg - recovery

↑ spleen, kidney weights - ≥100 µg/kg - recovery

↑ lung weights - 300 µg/kg ♂s - recovery

↑ adrenal weights - ≥1 µg/kg ♂s - recovery

#### Week 4 Kill

Spleen - hyalinization of white pulp germinal centers -  
≥100 µg/kg - recovery

Thymus - cortical atrophy - 300 µg/kg - recovery

BM - ↑ cellularity - 300 µg/kg - recovery

Liver - hepatocyte enlargement - 300 µg/kg ♂s - present at  
recovery (1/4 monkeys)

The NOEL was 1 µg/kg/day

#### 8. 13-Week SC Dose Toxicity Study of YM294 [rhIL-11] in Rats with an 8-Week Recovery Period

Species: Crj:CD(SD) rats (10-15/sex/grp)

Formulation: lyophilized

Dose Level: 0 (vehicle), 1, 10, 100, 1000 µg/kg/day [1 mL/kg]

Route/Duration: SC/13 weeks + 8-week recovery

Methods: Clinical signs, body weights, food & water consumption,  
clinical pathology, ophthalmoscopy, anti-IL-11 Abs, organ  
weights, and gross & microscopic pathology (control & 1000 µg/kg  
tissues & selected tissues from the lower dose grps) were  
evaluated.

#### Findings:

Hypoactivity, redness of extremities, prone position - ≥100 µg/kg  
- resolved

Edema of the extremities, rough haircoat - 1000 µg/kg - resolved

↓ BW gain, food consumption - 1000 µg/kg ♂s - resolved

Hemorrhage - right ocular fundus - 1/15 ♂s (1000 µg/kg) - week 13

**Abs** - [performed via ELISA at GI on 2-yr-old samples] samples seropositive for Abs at  $\geq 100 \mu\text{g/kg}$  by 13 weeks

↓ red cell mass, MCV, MCH; ↑ PLTs, WBCs -  $1000 \mu\text{g/kg}$  - week 13 - resolved

↓ HCT; ↑ PLTs -  $10 \mu\text{g/kg}$  ♂s - week 13 - resolved

↓ albumin -  $\geq 10 \mu\text{g/kg}$  ♂s &  $1000 \mu\text{g/kg}$  ♀s - week 13 - resolved

↑  $\alpha$  &  $\beta$  globulin; ↓ A/G ratio -  $1000 \mu\text{g/kg}$  - week 13 - resolved

↑  $\beta$  globulin -  $100 \mu\text{g/kg}$  - week 13 - resolved

↓ albumin, A/G ratio; ↑  $\beta$  globulin -  $10 \mu\text{g/kg}$  - week 13 - resolved

↓ albumin, total protein -  $1 \mu\text{g/kg}$  - week 13 - resolved

↑ megakaryocytes (myelogram) -  $1000 \mu\text{g/kg}$  ♂s

↓ urine volume; ↑ specific gravity -  $1000 \mu\text{g/kg}$  ♂s - weeks 6 & 12 - resolved

↑ urine protein - 2/15 ♂s ( $1000 \mu\text{g/kg}$ ) - present at recovery

↑ thyroid, kidney, spleen; ↓ prostate weight -  $1000 \mu\text{g/kg}$  - week 13 - resolved

#### Week 13 Kill

**Liver** - capsule thickening (9/10 ♂s); perivascular fibrosis (7/10 ♂s) -  $1000 \mu\text{g/kg}$

Perivascular fibrosis (3/10 ♂s) -  $100 \mu\text{g/kg}$

**Kidney** - enlargement of glomeruli & glomerular epithelial cells 8/10 ♂s ( $1000 \mu\text{g/kg}$ ) & 2/10 ♂s ( $100 \mu\text{g/kg}$ )

Eosinophilic droplets - epithelial cells - one ♂ at  $100 \mu\text{g/kg}$  & two ♂s at  $1000 \mu\text{g/kg}$

Hyaline casts - proximal tubular lumen - one ♂ at  $1000 \mu\text{g/kg}$

**Injection site(s)** - inflammatory cell infiltration - ↑ severity & incidence with ↑ dose

#### Recovery Kill

**Liver** - capsule thickening (3/5 ♂s); perivascular fibrosis (2/5 ♂s) -  $1000 \mu\text{g/kg}$

Perivascular fibrosis (2/5 ♂s) -  $100 \mu\text{g/kg}$

**Note:** findings were of a lower severity & incidence compared to the week 13 kill

**Kidney** - slight focal glomerulosclerosis; glomeruli adhered to Bowman's capsule; hyaline casts - proximal tubular lumen - (1/5 ♂s) -  $1000 \mu\text{g/kg}$

The NOAEL was  $10 \mu\text{g/kg/day}$

15. YM294: Toxicity to Cynomolgus Monkeys by Daily SC Administration for Up to 13 Weeks Followed by a Recovery Period  
Species: cyno monkeys (3-5/sex/grp)  
Formulation: lyophilized  
Dose Level: 0, 1, 10, 1000, 1000 µg/kg/day  
Route/Duration: SC/13 weeks + 4-week recovery (control & high dose only)

Methods: Clinical signs, BWs, appetite, clinical pathology (baseline and weeks 4, 8, 12 and recovery; and week 6 only for 1000 µg/kg), ophthalmoscopy, ECGs, anti-IL-11 Abs, PK samples, organ weights, and gross & microscopic pathology were performed.

Findings:

1/5 ♀s (1000 µg/kg) - killed moribund on day 2 of week 6  
Dosing of 1000 µg/kg monkeys halted - 3 ♂s & 1 ♀ killed on day 3 of week 6; with 2 ♂s & 3 ♀s designated as recovery

Hypoactivity, huddled posture, limb weakness/ataxia - 1000 µg/kg - starting week 2 into recovery

Joint stiffness/restricted movement, swollen arms/legs, SC nodules on arms/legs & under the chin, vocalization - 1000 µg/kg - weeks 5, 6 - recovery

Hypoactivity, huddled posture, limb weakness/ataxia - 100 µg/kg - starting week 2/3 - lower incidence/severity

Joint stiffness - one ♂/♀ - 100 µg/kg - weeks 5, 11-13

↓ BW (marked), appetite (slight) - 1000 µg/kg ♂s - recovery  
Bilateral peripapillary edema - 1000 µg/kg - week 6 - recovery

↓ HCT, HGB, RBCs - ≥10 µg/kg - week 4 (dose-related); 1000 µg/kg at week 6 - recovery

↑ retics - ≥100 µg/kg - weeks 4, 8, 12; 1000 µg/kg at week 6 - recovery

↑ hypochromasia, anisocytosis, rouleaux - ≥10 µg/kg (dose-related) - weeks 4, 8; 1000 µg/kg at week 6 - recovery

↑ PLTs - ≥100 µg/kg - weeks 4, 8, 12; 1000 µg/kg at week 6 - trend toward recovery

↑ PLTs - 10 µg/kg - weeks 4, 8, 12

[Dose-related in incidence]

Occasional giant PLTs were noted at ≥10 µg/kg - week 4

↑ fibrinogen - 10, 100 µg/kg - weeks 4, 8, 12; 1000 µg/kg - weeks 4, 6 - recovery

↓ PT - 100 µg/kg - week 4; 1000 µg/kg - weeks 4, 6 - recovery

↓ lymphocytes - 10, 100 µg/kg ♂s & Rx ♀s - week 4; 100 µg/kg - weeks 8, 12; 1000 µg/kg - weeks 4, 6 - trend toward recovery

↓ albumin, A/G ratio; ↑ globulin [ $\alpha$ -1,  $\beta$ ,  $\gamma$ -globulins] - 10, 100  $\mu\text{g/kg}$  - weeks 4, 8, 12; 1000  $\mu\text{g/kg}$  - weeks 4, 6 - recovery

↑ AlkP - 10  $\mu\text{g/kg}$  - weeks 4, 8, 12; 1000  $\mu\text{g/kg}$  - weeks 4, 6 - recovery

↓ AST, ALT, LDH, CPK; ↑ sorbitol dehydrogenase, triglycerides - 100  $\mu\text{g/kg}$  - weeks 4, 8, 12; 1000  $\mu\text{g/kg}$  - weeks 4, 6 - recovery

↓ Fe -  $\geq 10$   $\mu\text{g/kg}$  - weeks 4, 8, 12; 1000  $\mu\text{g/kg}$  - weeks 4, 6 - recovery

↑ heart weights - 10, 100  $\mu\text{g/kg}$  - week 13; 1000  $\mu\text{g/kg}$  - week 6 - no recovery

↑ liver weights - 10, 100  $\mu\text{g/kg}$  - week 13; 1000  $\mu\text{g/kg}$  - week 6 - recovery

↓ thymus weights - 10, 100  $\mu\text{g/kg}$  - week 13; 1000  $\mu\text{g/kg}$  - week 6 - recovery

↑ kidney weights - 10, 100  $\mu\text{g/kg}$  - week 13; 1000  $\mu\text{g/kg}$  - week 6 - recovery

#### Histopathology - 1000 $\mu\text{g/kg}$ - week 6 kill

Bones/joints - periosteal hyperostosis [femur, sternum, & hip, shoulder, ankle joint]; fibrosis [joint capsule, ligaments, & adjacent muscles to various joints] - 3/3  $\sigma$ s, 2/2  $\text{f}$ s

Injection sites/SC tissue - fibrosis - 3/3  $\sigma$ s, 2/2  $\text{f}$ s

BM - hypertrophy of megakaryocytes; ↓ adipocyte numbers;

↑ prominent hematopoietic marrow

Thyroid - minimal follicular epithelial hypertrophy - 3/3  $\sigma$ s, 2/2  $\text{f}$ s

Thymus - involution - 3/3  $\sigma$ s, 2/2  $\text{f}$ s

Liver - vacuolar inclusions in periportal hepatocytes

#### Week 13 kill

Bones/joints - periosteal hyperostosis [femur, sternum, & hip, shoulder, ankle joint]; fibrosis [joint capsule, ligaments, & adjacent muscles to various joints] - 10  $\mu\text{g/kg}$  (2  $\sigma$ s, 1  $\text{f}$ );

100  $\mu\text{g/kg}$  (3/3  $\sigma$ s, 3/3  $\text{f}$ s)

Injection sites/SC tissue - fibrosis -  $\geq 1$   $\mu\text{g/kg}$  (dose-related)

BM - hypertrophy of megakaryocytes, ↓ adipocytes, ↑ prominent hematopoietic marrow - 100  $\mu\text{g/kg}$

Thyroid - follicular epithelial hypertrophy -  $\geq 10$   $\mu\text{g/kg}$  (dose-related)

Thymus - involution - 100  $\mu\text{g/kg}$

#### 13-week recovery kill - 1000 $\mu\text{g/kg}$ only

Bones/joints - ↑ thickness of cortex, with ↑ osteocytes in subperiosteal region [lesion remodeling] - 2/2  $\sigma$ s, 3/3  $\text{f}$ s

BM - ↑ prominent hematopoietic marrow

Thyroid - follicular epithelial hypertrophy - 1/3  $\text{f}$ s

The NOAEL was 1  $\mu\text{g/kg/day}$ .

**Comments:**

- [Per the sponsor] The possible production of ferritin, an acute phase protein, by IL-11, resulting in greater iron storage capacity in the tissues and less iron in the plasma. IL-11 also suppresses adipogenesis - as noted in the bone marrow - thus could indirectly affect triglyceride levels.
- [Per the sponsor] The clinical manifestation of the alterations in joint morphology was very apparent during weeks 5 & 6 in the 1000 µg/kg monkeys.
- IL-11 has been found to act in cultures of bone marrow cells to enhance parathyroid hormone-induced or vitamin D-induced osteoclast development (Passeri, G., et al., IL-11: A new cytokine with osteoclastogenic and bone resorptive properties and a critical role in PTH- and 1,25(OH)<sub>2</sub>D<sub>3</sub>-induced osteoclast development, J. Bone Min. Res (sup):S110, 1992). These investigators also found that IL-11 [produced by osteoblasts] could activate osteoclasts, seen in the enhanced ability to resorb bone. Administration of high amounts of exogenous IL-11 could alter the osteoblast-osteoclast balance, possibly leading to the hyperostosis and bone remodeling noted in the monkeys.
- The increase in the heart weights may be associated with the expanded plasma volume that results from dosing with IL-11.
- The AlkP changes may be reflective of the bone changes, but AlkP isoenzymes were not measured.
- IL-11 is produced by fibroblasts.

**Reproduction/Teratology Studies****List of Studies:**

Note that the dates presented with each study are the dates the report was issued, not the date of study completion.  
The list of studies are numbered in the order in which they appear in the submission.

1. Reproductive and Developmental Toxicity Study in Rats Administered YM294 SC During Fetal Organogenesis (Seg. II); study #KI93102; performed at Ina Research, Inc., Japan (per GLP); lot #TOX015 (lyophilized - 0.3 M Glycine/20 mM L-Histidine); 9/94

2. Reproductive and Developmental Toxicity Study of YM294 Administered SC to Rats Prior to and in the Early Stages of Pregnancy (Seg. I); study #KI94032; performed at Ina Research, Inc., Japan (per GLP); lot #WN2944A (lyophilized - 0.3 M Glycine/0.01 M Sodium Phosphate); 8/95

3. Reproductive and Developmental Toxicity Study of YM294 Administered SC to Female Rats Prior to or in the Early Stages of Pregnancy; study #KI95033; performed at Ina Research, Inc., Japan (per GLP); lot #WN2944A (lyophilized - 0.3 M Glycine/0.01 M Sodium Phosphate); 11/95

### Reproduction/Teratology Studies

#### 1. Reproductive and Developmental Toxicity Study in Rats Administered YM294 SC During Fetal Organogenesis (Seg. II)

Species: Crj:CD(SD) rats (36 ♀s/group)

Formulation: lyophilized

Dose Level: 0, 10, 100, 1000 µg/kg/day

Route/Duration: SC/GD 7-17

Kill: C-sections on [gestation day] GD 20 (24 ♀s/group) & [lactation day] LD 22 (12 ♀s/group)

#### Methods:

F<sub>0</sub> - Clinical signs, body weights, food consumption, and gross pathology

On GD 20 - Gross observations + uterine examination

On LD 22 - Gross observations + uterine examination

F<sub>1</sub> - Fetuses - sex, weight, external, visceral, skeletal abnormalities

F<sub>1</sub> - Pups - live births, sex, weight, external, visceral, skeletal abnormalities, viability & weaning indices, physical development, behavioral indices, mating indices

killed on GD 20 - Gross observations + uterine examination

F<sub>2</sub> - Fetuses - sex, weight, external abnormalities

#### Findings:

F<sub>0</sub> - 100 µg/kg - one ♀ died on GD 23 - hemorrhages in stomach; enlarged lungs & foamy fluid in bronchi - all dead fetuses appeared normal developmentally

100 µg/kg - dyspnea, hypoactivity, in prone position - GD 15-17

1000 µg/kg - ataxia, dyspnea, hypoactivity, in prone position - GD 14-17

The signs were noted postdose and resolved by 70 minutes postdose.

↓ food consumption - GD 8-18 - 1000 µg/kg  
Gross pathology (GD 20 & LD 22) - no abnormalities

One litter died on LD 1 - 1000 µg/kg

F<sub>1</sub> - Fetuses - ↓ body weight; ↓ number of ossified sacral/caudal vertebrae - 1000 µg/kg

F<sub>1</sub> Pups - no abnormalities

F<sub>2</sub> Fetuses - no abnormalities

The NOEL was 10 µg/kg/day for dam toxicity; 100 µg/kg/day for F<sub>1</sub> teratology; and 1000 µg/kg/day for perinatal and postnatal development

## 2. Reproductive and Developmental Toxicity Study of YM294 Administered SC to Rats Prior to and in the Early Stages of Pregnancy (Seg. I)

Species: Crj:CD(SD) rats (22/sex/group)

Formulation: lyophilized

Dose Level: 0, 10, 100, 1000 µg/kg/day

Route/Duration: SC

♂s - 63 days prior to mating, during mating (14 days), up to the day prior to kill

♀s - 14 days prior to mating, during mating (14 days), through GD 7

Kill: mated ♀s - C-sections on GD 20

unmated ♀s - killed 11 days after the end of the mating period

♂s - killed after GD 7 or killed 11 days after the end of the mating period with the unRx ♀s

### Methods:

F<sub>0</sub> ♀s - Clinical signs, body weights, food consumption, estrous cycles, and gross pathology

On GD 20 - Gross observations + uterine examination

F<sub>0</sub> ♂s - Clinical signs, body weights, food consumption, and gross pathology

F<sub>1</sub> - Fetuses - sex, weight, external, visceral, skeletal abnormalities

### Findings:

F<sub>0</sub> ♂s - 1000 µg/kg - two ♂s died on day 9 & 10 - hypoactivity; purple discoloration of eyeballs and extremities; dyspnea; subdural bleeding

One control ♂ died on day 128 with similar findings

100 µg/kg - hypoactivity, in prone position - days 9-13  
1000 µg/kg - dyspnea, hypoactivity - days 9-13; rough haircoat - starting on day 37

↓ BW gain - 1000 µg/kg - days 15-43 - remained low throughout the study

↓ food consumption - days 1-22 - recovery by day 43

No gross lesions noted

F<sub>0</sub> ♀s - 1000 µg/kg - hypoactivity, in prone position - days 8-12

100 µg/kg - hypoactivity, dyspnea, in prone position - days 11-13

↓ BW - GD 7-10 - 1000 µg/kg

↓ food consumption - day 1- GD 10 - 1000 µg/kg

No gross lesions noted

Prolonged estrous cycles -

Length of cycle >6 days (dose-related) [compared to within 5 days for control] - ≥100 µg/kg

Reduced number of cycles - ≥100 µg/kg (dose-related)

No abnormalities in the mating/fertility index

No differences in the number of corpora lutea

UnRx ♀s - no abnormalities

No abnormalities in the mating/fertility index

No abnormalities in the uterine contents

F<sub>1</sub> - Fetuses - ↓ live fetuses/↑ early deaths - ≥100 µg/kg

No Rx-related anomalies/variations

The NOEL was 10 µg/kg/day for parental reproductive function and 10 µg/kg/day for fetal toxicity

### 3. Reproductive and Developmental Toxicity Study of YM294

Administered SC to Female Rats Prior to or in the Early Stages of Pregnancy

Species: Crj:CD(SD) rats (22/sex/group)

Formulation: lyophilized

Dose Level: 0, 1000 µg/kg/day

Route/Duration: SC/14 days prior to mating, during mating (14 days) until copulation (GD 0) or from GD 0-7

Kill: mated ♀s [with unRx ♂s] - C-sections on GD 15

**Methods:**

F<sub>0</sub> ♀s - Clinical signs, body weights, food consumption, estrous cycles, clinical pathology (GD 8 & 15), and gross pathology  
On GD 15 - Gross observations + uterine examination

F<sub>1</sub> - Embryos - implantation loss, deaths, BWs

**Findings:**

F<sub>0</sub> ♀s treated prior to mating to GD 7 - hypoactivity/prone position - 19/22 ♀s - days 8-11

↓ BW - day 15, GD 0-7

↓ food consumption - days 1-15; ↑ food consumption - GD 7-11

Prolonged estrous cycles -

Length of cycle > 6 days [compared to within 5 days for control] for 9/22; with 2/22 showing one or no cycles

↑ WBCs, lymphocytes, cholesterol, total protein (α1 & β-globulin), albumin, Fe binding capacity; ↓ AST, ALT - GD 8

GD 15 - chemistry changes similar to those on GD 8

↓ corpora lutea; ↓ gravid uterine weight

No gross lesions noted

F<sub>1</sub> - ↓ implantations, live embryos, embryo weight

No difference in pre-implantation loss, placental weight

F<sub>0</sub> ♀s treated GD 0-7 - hypoactivity/prone position - 5/22 ♀s

↓ BW - GD 11-15

↓ food consumption - GD 0-11

↓ red cell mass, PLTs, cholesterol, albumin, Fe binding capacity, ↓ AST, ALT; ↑ WBCs, lymphocytes, BUN, total protein - GD 8

↓ PLTs; ↑ WBCs, total protein (α1 & β-globulin), Fe binding capacity - GD 15

No gross lesions noted; ↓ gravid uterine weight

F<sub>1</sub> - ↓ live embryos, embryo weight

No difference in implantation number, pre-implantation loss, placental weight

Administration of 1000 µg/kg/day of IL-11 prior to, and in the early onset of pregnancy resulted in ↓ live embryos

### Mutagenicity Studies

#### List of Studies:

Note that the dates presented with each study are the dates the report was issued, not the date of study completion.  
The list of studies are numbered in the order in which they appear in the submission.

1. Human Lymphocyte Metaphase Analysis; study #YMI 88/930239; performed at Huntingdon Labs (per GLP); lot #TOX-012 (lyophilized); 6/93
2. Mammalian Cell Mutation Assay; study #YMI 89/930206; performed at Huntingdon Labs (per GLP); lot #TOX-012 (lyophilized); 6/93
03. Mouse Micronucleus Test; study #YMI 90/930582; performed at Huntingdon Labs (per GLP); lot #TOX-012 (lyophilized); 6/93

#### In Vitro Mammalian System

##### 1. Human Lymphocyte Metaphase Analysis

Batch #: TOX 012

Cell: Human peripheral lymphocytes +/- S9

Dose Level: 0, 250, 500, 1000 µg/mL

Findings: No significant increases in either the proportion of aberrant cells (clastogenic activity) or in the number of polyploid cells was found. No significant decreases in the mitotic index occurred.

##### 2. Mammalian Cell Mutation Assay

Batch #: TOX 012

Cell: Mouse lymphoma L5178Y cells +/- S9

Dose Level: 0, 30-1000 µg/mL

Findings: Cells cultured without S9 mix, resulted in no significant increases in the mutant frequency. A statistically significant increase in the mutant frequency was observed with S9 mix + 250 µg/mL of IL-11, but the increase was small and was not present in the duplicate test run. Final conclusion - no increases in forward mutant frequency [from the thymidine kinase locus to the thymidine kinase deficient genotype] noted.

In Vivo Mammalian System**3. Mouse Micronucleus Test**

Batch #: TOX 012

Species: CD-1 mice

Dose Level: 0, 2.5, 5.0, 10.0 mg/kg

Route: IP

**Findings:** No evidence of systemic toxicity after 24, 48, 72-hour observation/sampling periods (BM smears). The polychromatic/normochromatic (PCE/NCE) RBC ratio was not affected. No indication of chromosome or spindle-function damage [as exhibited by the induction of micronuclei] was noted. No significant change in the number of micronuclei (in 1000 polychromatic RBCs) was noted.

**CONCLUSION:**

The proposed clinical indication for IL-11 is in the prevention of chemotherapy-induced thrombocytopenia & the reduction of the need for platelet transfusions in patients with non-myeloid malignancies. The proposed package insert submitted by the sponsor states that 50 µg/kg/dose NEUMEGA® is to be SC injected once daily 6-24 hours after chemotherapy completion for 10-21 days per treatment course.

Rats and monkeys constituted the primary species used in the performance of the toxicology studies [IL-11 was pharmacologically active in these species]. The single dose IV bolus injection studies of 10 mg/kg IL-11 in rats resulted in transient hypoactivity and auricular redness. A single IV injection in primates up to 10 mg/kg resulted in expected exaggerated pharmacological effects, such as increased PLTs, globulin, fibrinogen, & AlkP; extramedullary hematopoiesis; and decreased red cell mass & albumin.

Repeated dose studies performed in rats and cynomolgus monkeys have ranged from 2 to 13 weeks in duration with SC injection of IL-11 doses from 1 to 1000 µg/kg/day. The rats have displayed significant adverse effects at levels ≥100 µg/kg/day, which have included hypoactivity; elevated plasma fibrinogen; anemia; increased spleen & liver weights; development of anti-IL-11 Abs; fibrosis at injection sites & in the liver; and a dose-related increase in the frequency and/or severity of the thickening of the femoral and tibial growth plates. Similar, but less severe, effects were seen at 10 & 30 µg/kg/day. The desired pharmacological effect of elevated PLTs was noted at IL-11 doses of ≥10 µg/kg/day. With the exception of the bone changes, all

effects have been fully reversible. After a single SC dose of 100  $\mu\text{g/kg/day}$  of IL-11 in rats, an approximate  $\text{AUC}_{0-\infty}$  of 24% and a  $\text{C}_{\text{max}}$  of 92% of the mean values calculated in healthy adult males given a single SC injection of 50  $\mu\text{g/kg}$  were obtained.

The monkeys have followed a pattern similar to the rats, exhibiting significant effects at IL-11 doses of  $\geq 100 \mu\text{g/kg/day}$ . Findings noted have included swollen limbs; subcutaneous nodules; vocalizations; weight loss; injection site fibrosis; development of anti-IL-11 Abs; anemia; elevated acute phase proteins; increased liver, kidney, spleen, & heart weights; periosteal hyperostosis of bones & tendons; and joint capsule fibrotic responses. In addition, papilledema (optic nerve head edema), with no microscopic correlate has been noted in monkeys dosed at 1000  $\mu\text{g/kg/day}$ . All findings were fully reversible, and partial recovery of the bone changes by bony remodeling was noted after 13 weeks of recovery. The desired pharmacological effect of elevated PLTs was noted at IL-11 doses of  $\geq 10 \mu\text{g/kg/day}$ . After a single SC dose of 100  $\mu\text{g/kg/day}$  of IL-11 an approximate  $\text{AUC}_{0-\infty}$  of 159-656% and a  $\text{C}_{\text{max}}$  of 203-1333% of the mean values calculated in healthy adult males given a single SC injection of 50  $\mu\text{g/kg}$  were obtained. After a single SC dose of 10  $\mu\text{g/kg/day}$  of IL-11, an approximate  $\text{AUC}_{0-\infty}$  of 12-21% and a  $\text{C}_{\text{max}}$  of 17-28% of the mean human male values (after a dose of 50  $\mu\text{g/kg}$ ) were obtained.

Based on the toxicology studies, it can be concluded that 1) acute phase protein induction [fibrinogen, complement C3 & C4, haptoglobin, properdin factor B, etc...] is correlated with the effect of IL-11 on hepatocytes and 2) decreases in albumin, iron, total iron binding capacity, and calcium and increases in serum globulins & liver weights are also reflective of the acute phase response. These changes have been noted in clinical trials.

In addition, IL-11 administration to animals and humans has resulted in plasma volume expansion. In animals findings of anemia, conjunctival edema, swollen limbs, dilutional decreases in serum chemistry values; increased kidney & heart weights; and papilledema have reflected this expansion. This reversible effect has also been noted in clinical trials and has been managed with the use of diuretics.

Some incidences of atrial cardiac arrhythmias and other cardio-pulmonary events have been displayed in clinical trials. Such effects have not manifested in the preclinical safety pharmacology and toxicology studies performed.

Of significance is the apparent effect of IL-11 on the musculo-skeletal system. Rats - dosed at a time when they were rapidly growing - displayed dose-related growth plate thickening after 4 weeks of exposure to  $\geq 100 \mu\text{g/kg/day}$  of IL-11, with a trend towards recovery after 4 weeks of nontreatment. Cynomolgus

monkeys displayed a dose-related periosteal hyperostosis & fibrosis of joint capsules & tendon insertions at  $\geq 100 \mu\text{g/kg/day}$  for 2 to 13 weeks and at  $\geq 10 \mu\text{g/kg/day}$  at 13 weeks, with a trend towards recovery. Clinically, the monkeys also showed hypoactivity, swollen limbs, subcutaneous nodule formation near affected bones, and elevated AlkP values.

In addition, IL-11 has been found to act in cultures of bone marrow cells to enhance parathyroid hormone-induced (PTH) or vitamin D-induced osteoclast development (Passeri, G., et al., IL-11: A new cytokine with osteoclastogenic and bone resorptive properties and a critical role in PTH- and  $1,25(\text{OH})_2\text{D}_3$ -induced osteoclast development. J. Bone Min. Res (sup):S110, 1992). These investigators also found that IL-11 could activate osteoclasts, seen in the enhanced ability to resorb bone. IL-11 production is also induced from osteoblasts when stimulated with known bone-forming stimuli such as IL-1, TGF- $\beta$ , & PTH (Girasole, G., et. al., IL-11: A new cytokine critical for osteoclast development. J. Clin. Invest. 93:1516-1524, 1994). It is postulated that one of the pleiotropic effects of IL-11 is the involvement with bone turnover. One pediatric patient has displayed an asymptomatic periosteal reaction. The sponsor is aware of this adverse effect and has provided a notation in the package insert under the "pediatric use" heading. It is recommended that the label wording regarding this finding in animals and humans be revised and highlighted.

Segment I and II reproductive toxicity studies in rats using the histidine/glycine lyophilized formulation of IL-11. Administration of IL-11 from prior to mating to gestation day 7 (Segment I) revealed a NOEL of  $10 \mu\text{g/kg/day}$  for female  $F_0$  reproductive function, as well as fetal toxicity. At IL-11 doses of 100 and  $1000 \mu\text{g/kg/day}$ , females displayed prolonged estrous cycles, decreased numbers of live fetuses, and increased numbers of early fetal deaths. Doses of  $100 \mu\text{g/kg/day}$  or greater resulted in transient hypoactivity and dyspnea in the treated  $F_0$  females and males. No effects on male reproduction parameters were noted.

SC injection of  $10 \mu\text{g/kg/day}$  of IL-11 during the period of organogenesis (Segment II) in rats did not result in any maternal, fetal, or postnatal abnormalities. SC doses of 100 and  $1000 \mu\text{g/kg/day}$  resulted in maternal toxicity. Doses of  $1000 \mu\text{g/kg/day}$  resulted in retarded development of the  $F_1$  fetuses, to include low body weights and delayed ossification of the sacral and/or caudal vertebrae, but did not result in any long term behavioral or developmental abnormalities in  $F_1$  offspring nor any teratological effects in  $F_2$  fetuses.

No mutagenic potential was exhibited via in vitro or in vivo mammalian systems. IL-11 did not stimulate tumor colony forming units from primary tumor cells obtained from various surgically excised human tumors.

At the time of the mid-cycle review (held on 4/30/97), several questions were brought up that related to the preclinical data generated by Genetics Institute:

1. Study C9416 - Are data available on the use of IL-11 in multidose chemotherapy cycles? Refer to animal study #21 under the pharmacology section - "Thrombopoietic Activity of IL-11 in a Novel Myelosuppressed Nonhuman Primate Model" - in which carboplatin (days 1-3) + IL-11 (125  $\mu\text{g/kg/day}$  starting on either day 1 or day 4) was injected for cycle 1. Cycle 2 was initiated approximately one month later (IL-11 starting one day following chemotherapy [days 29-31]). G-CSF was SC injected at 10  $\mu\text{g/kg/day}$ , along with G-CSF (10  $\mu\text{g/kg/day}$ ).

In Cycle 1, PLT counts for 5/6 IL-11 monkeys did not go below 20,000/ $\mu\text{L}$ , regardless of the treatment schedule [one animal dosed starting in day 1 experienced notable thrombocytopenia]. However, 5/6 control monkeys were at or below this level. In addition, IL-11 treatment showed a range of 2-5 days [day 1 dosing commencement] & 0-3 days [day 4 dosing commencement] for PLT recovery to  $\geq 50,000$  cells/ $\mu\text{L}$ , while controls displayed a range of 3-8 days.

In Cycle 2, 3/3 monkeys injected with IL-11 on day 1 (Cycle 1) & 1/3 monkeys injected with IL-11 on day 4 (Cycle 1) were below 20,000/ $\mu\text{L}$ . Controls were at this nadir in 4/6 monkeys. PLT recovery was notably slower in the day 1-cycle 1 dosing regimen compared to the day 4-cycle 1 dosing regimen. Additionally, the time to recovery to  $\geq 100,000$  cells/ $\mu\text{L}$  post-cycle 2 was increased for the IL-11 animals compared to controls (see figures). The day 1-cycle 1 monkeys exhibited a trend toward longer neutrophil recovery times, displayed in cycle 2, compared to the control & day 4-cycle 1 monkeys. Red cell indices were lower for the IL-11 monkeys in cycle 1, but appeared similar to controls in cycle 2. A total of 1/6 control, 2/3 day 1-cycle 1, and 2/3 day 4-cycle 1 animals required RBC transfusions.

2. Study C9416 - Are data available on the function of the PLTs produced by IL-11? Refer to animal study #14 under the pharmacology section - "Effects of SC Administered IL-11 on PLT Reactivity & PLT Ultrastructure in Nonhuman Primates" - in which

PLTs from normal cynomolgus monkeys injected at 125  $\mu\text{g/kg/day}$  for 7 days were increased by 156% from baseline (day 11). PLTs retained a normal discoid shape (determined by ultrastructural analysis). Via P-selection upregulation in response to thrombin (PLT reactivity), a slight increase in PLT reactivity after 4 days of IL-11 was noted - correlating with new PLTs in the circulation.

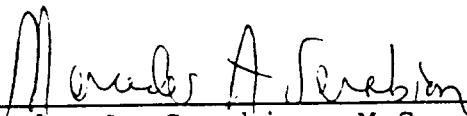
In the toxicity studies in monkeys & rats, an increase in whole blood PLT aggregation slopes were noted, possibly due to the increased number of PLTs generated. Note that in a 28-day toxicity study in monkeys, one 1  $\mu\text{g/kg}$  male with high PLT counts, killed moribund on day 29, had multiple pulmonary thrombi eliciting edema due to venous congestion. Note that in another study deaths occurred in IL-11 exposed normal & myelosuppressed beagle dogs from hemorrhagic pneumonia. The lungs contained vascular congestion & hemorrhage. There exists a potential relationship between IL-11-induced thrombocytosis and these hemorrhages.

Anticipating the concurrent administration of G-/GM-CSF with IL-11, the sponsor performed a short study in monkeys, in which IL-11 alone, G-CSF alone, GM-CSF alone, or the various combination of growth factors were injected for 14 days. Expected pharmacologic changes included  $\uparrow$  WBCs, neutrophils, monocytes, & lymphocytes in the IL-11 + G-/GM-CSF groups and in the G-/GM-CSF alone groups and anemia and increased PLTs & RBC distribution width in all IL-11 grps (alone & combination). The day 1 AUC &  $C_{\text{max}}$  values for the IL-11 alone or combination groups were similar. However, the day 14 AUC &  $C_{\text{max}}$  for IL-11 + G-CSF were slightly higher compared to IL-11 alone or IL-11 + GM-CSF. The significance of these data are questionable, as much intra- and interanimal variation existed and the sample size was small (2-4 monkeys per group). Of note is that there was no apparent unexpected effects on the hematopoietic parameters evaluated nor the toxicology of the various combinations of agents.

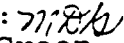
The preclinical data adequately support use of the product, NEUMEGA®, for the dosage regimen specified by the sponsor. The package insert will be modified to better reflect the preclinical data [to be included as an attachment to this review]. In addition, many cover pages in the toxicology study reports were submitted unsigned. The sponsor will be requested to submit appropriate signature pages of all toxicology study reports, in addition to any amendments/revisions to the reports that were not included. This request will be made in the mid-cycle review letter.

## COMMUNICATION (for mid-cycle review letter):

"Many cover pages in the toxicology study reports were submitted without final signatures and dates, as per Good Laboratory Practice requirements. Please submit appropriate signature pages for each toxicology study report, in addition to any amendments/revisions that you have made to the reports since their submission to FDA."

 5/27/97  
Mercedes A. Serabian, M.S., D.A.B.T., Toxicologist

**Key Words:** IL-11; NEUMEGA®; platelets; chemotherapy; transfusion; myelosuppression; ophthalmology; acute phase response; dilutional anemia; femoro-tibial joint; osteoclasts; bone resorption

concurrences:  7/30/97  
OTRR/C, P-T/MGreen

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OTRR/C, P-T/MGreen  
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